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THE OCCURRENCE IN NATURE OF CERTAIN YEAST-LIKE FUNGI WITH REFERENCE TO THEIR POSSIBLE PATHOGENICITY IN THE HIGHER ANIMALS

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INTRODUCTION

The biologist who studies the diseases of man and the higher animals occasionally discovers a fungus that may have importance in connection with a particular malady. At first the association of fungi in animal tissues received only a passing notice, but later research indicated that in certain diseases fungi may play an important rôle. These fungi not only affect animals as a result of their parasitic growth, but also as a toxin if consumed in large quantities on infected foods.

Up to the present time there have appeared only two general reference texts dealing with the fungous parasites of man and the higher animals. Gedoelst ('02) in 'Les champignons parasites' and Plaut ('03) in 'Die Hyphenpilze oder Eumyceten' discuss in a general way the subject of fungous parasites of animals, more particularly from a medical point of view. Both writers give historical accounts of the discoveries and investigations on this subject from the time fungi were first recognized as agents in the production of disease. These books, published more than ten years ago, are the only general treatises that have appeared abroad, and there has not appeared any general work of American origin on these fungous parasites of the higher animals. Nevertheless, for the past few years there has been active investigation in this field, and a large amount of information has been published concerning more particularly the physiological relations of parasite and host.

The purpose of the present investigation is twofold. In the first place, it seems advisable to learn from the literature on

the subject of animal pathology the relative importance of fungi as producers of diseased conditions in the higher animals. The arrangement of this material will be in taxonomic sequence with respect to the fungi. In the second place, this paper will consider the distribution in nature of the known pathogenic fungi, and also will give the results of the author's experiments to determine whether there are any organisms among the very large number of saprophytic wild yeasts that might prove pathogenic when introduced into the bodies of animals.

Quite recently Loeb, Moore, and Fleisher ('13) obtained a culture of a yeast-like organism from an infected sarcoma which had developed in the tissues of a man sixty-two years of age. After the operation was performed the tumor was removed to the laboratory, and was sterilized by searing the surface with a heated spatula. Small pieces of the tissue, removed from the interior with sterilized instruments, were placed in tubes of sterilized sugar solution; and after about twenty-four hours the culture liquid became turbid, due to the presence of a yeast-like fungus. Inoculation experiments on animals demonstrated that the organism was very pathogenic. This fungus was studied in the laboratory at the Missouri Botanical Garden by Professor George T. Moore. Its accidental occurrence in cancer and the pathogenic action on animals suggested the investigation undertaken in this paper.

Many of the so-called pathogenic fungi were carefully studied before their importance as disease-producing organisms was recognized. Other parasitic fungi were discovered only after the characteristic disease had been known for centuries, and it remained for modern methods of investigation to determine the true nature of the disease. A brief historical survey, consequently, will develop important and interesting facts bearing upon the relations of scientific research to the advancement of the study of microbiology.

The pathogenic rôle of the fungi has been much discussed. The first authors considered them as saprophytes which were developed in special circumstances, more often in a preëxisting lesion. Others, on the contrary, admit that these

moulds can establish themselves on the surface of sound mucus, develop, and extend their filaments gradually and give rise to a special disease. Weichselbaum ('78) took the view opposing Virchow, in that the mould *Aspergillus* may infect tissues that were not previously diseased.

HISTORICAL

The first report of a fungus occurring in the human body is that made in 1736 by Horn and Degener (cited by Virchow, '56) who observed a mould growth in gangrenous places on a man's foot. Heusinger in 1826 (cited by Virchow, '56), observed fungous elements in the fresh scales of a ring-worm lesion and considered this find as worthy the attention of botanists. This hint, however, remained unnoticed until the Italian, Bassi, in 1837 (cited by Plaut, '03), discovered that one type of silkworm disease was caused by a fungous parasite. Thereupon Schonlein (cited by Plaut, '03) was stimulated to investigate the infectious scalp diseases of man, with the result that in 1839 he found a hyphomycete to be the cause of the disease known as favus. This is the first incident in which a hyphomycete was known to be the cause of a human disease. At about the same time Langenbeck and Berg discovered the organism which is the cause of thrush.

An interesting observation, but of a questionable nature, is that made by Olsen in 1886 (cited by Guéguen, '05) in support of the pathogenicity of *Sterigmatocystis nigra*. Olsen reports that on removing the bandage from a large flesh wound of a man he found a black mould that seemed to have penetrated the epidermis. This fungus, upon examination under the microscope, presented all the appearances of *S. nigra*. The wound, after being washed with a sublimate solution, was redressed with an iodoform gauze and overlaid with sublimate and a layer of peat. Upon removing this bandage a week later, there was evidence of an extensive development of this same fungus. Brefeld confirmed Olsen's determination, although the spores and mycelia, when transferred to a culture solution, failed to germinate.

While the occurrence of moulds in the lungs of birds was known in 1815, it was not discovered in man until 1847. The first notice of a real pneumonomycosis caused by *Aspergillus* was made by Baum, Litzmann, and Eichstett (cited by Plaut, '03) from sections of a diseased lung taken from a woman's body shortly after death. The first scientific description of such a case is that by Virchow ('56) who, on three occasions, found in human bodies such gangrenous colonies as could be, he considered, easily differentiated, by the absence of an odor, from ordinary inflammations of the lung. The fresh lung colonies were of a hemorrhagic nature and, according to Virchow, of secondary importance. Descriptions of the parasite as found in similar instances by Fürbringer ('76) indicate that the fungus may have been a species of *Mucor*.

French authors took the view that a primary lung mycosis exists in man and may quickly change into conditions of tuberculosis; but that the lung mycosis may also be complicated with tuberculosis, whereupon the distinction becomes very difficult. Chantemesse ('91) discovered similar conditions in the men who care for pigeons, and whose lungs become diseased in consequence of their vocation. These men masticate the bird food, which consists of grain, and the young birds eat directly from the caretaker's mouth. Infection with fungous spores doubtless takes place during this feeding process. The development of the disease resembles entirely a chronic lung tuberculosis. Histologically, the lesions in birds and mammals resemble in their structure the lesions of the tubercle bacillus of Koch.

The first observation of a fungus in the ear was reported by Mayer in 1844 (cited by Plaut, '03), who found a fungous mass (possibly *Aspergillus*) in the ear of an eight-year-old girl. Pacini, in 1851, and Grove, in 1857 (cited by Plaut, '03), reported *Sterigmatocystis nigra*, and Cramer, in 1859 (cited by Plaut, '03), reported *S. nigra (antacustica)* as occurring in the ear. Schwatze, von Wreden, and Bezold (cited by Plaut, '03) upheld the parasitic theory of this fungus. More recently Hatch and Row ('00), in India, where

ear mycosis is a very virulent disease, reported the occurrence of *Sterigmatocystis nigra*, *Aspergillus viridescens*, *A. fumigatus*, *A. albus*, *A. glaucus*, and *A. flavescens*. The ear undergoes a serious catarrh, less frequently conditions of purulent discharge. The fungi, *Aspergillus fumigatus* and *Sterigmatocystis nigra*, investigated by Siebenmann ('89), grow poorly, if at all, on the normal epidermis.

There is little mention of fungi appearing in diseases of the nose. Schubert ('85) reported the occurrence of *Aspergillus fumigatus* on surface lesions in the nose. In another incident, a distiller was so afflicted that both the lower and middle nasal cavities were filled with a gray-green secretion of a characteristic odor. A microscopic investigation revealed a hyphomycete with long, single-celled, sickle-shaped conidia. No cultures were made, but Ferdinand Cohn pointed out the similarity of this fungus to *Isaria*, certain species of which are known to be parasitic on insects.

Ceratomycosis is of rare occurrence, and only a few cases have been described. This condition is usually brought about by means of an injury from a falling body infected with fungous spores. The first incident is that reported by Leber ('82), in which a farmer forty-five years old, working on a threshing-machine, was struck in the eye by an oat scale. Berliner and Uhthoff ('83) reported a ceratomycosis caused by a falling pear striking a farmer in the eye. Fuchs ('94) mentions a case of inflammation in the right eye of a miller fifty-three years old and sick with fever, the condition being apparently due to an injury and a later infection with *Aspergillus fumigatus*.

The above-mentioned investigators dealt only with localized diseases of a single tissue. Zenker, in 1861 (cited by Plaut, '03), stated that what had originally occurred as thrush on the mucous membrane of a man, had produced metastases in the brain in the form of multiple abscesses. Grohe, in 1870, injected the spores of moulds into the veins of rabbits and presumably obtained metastases of the inner organs. Michailow ('11) mentions the occurrence in two cases of Asiatic cholera, of fungus-like elements in the cen-

tral nervous system, but he obtained no cultures of the organism.

The spores of fungi, introduced by injection into the blood-vessels, are carried by the blood into all parts of the body. They do not germinate in the blood current itself, but only in certain organs of the animal into which they are conveyed by the blood. The living organs show different degrees of liability to the attack of the fungus, especially when the spores are injected in small quantities. Lichtheim ('82) arranges the susceptibility of the various organs to *Mucor* in the following descending series: kidneys, Peyer's patches, mesenteric glands, spleen, marrow, and the liver. After the death of the animal there is no difference in the rate of germination and development of the fungus in the organs. The development of the fungus is attended by characteristic local derangements, and these produce disturbances of the general health. Sticker ('00) asks the question whether or not the large amount of carbon dioxide formed by the development of the fungus does not cause the injurious effects on the animals. Others believe that a fermentative action plays an important rôle. Spontaneous *Aspergillus* and *Mucor* mycoses in internal organs removed from the direct access of air are, to say the least, a doubtful occurrence.

Grawitz ('77) attempted to produce infection in dogs and rabbits by inoculations with spores of *Mucor Mucedo*, *M. racemosus*, and *Rhizopus nigricans*. None of the two hundred animals used died from the effects of these treatments. Experiments of Lichtheim ('82) and Lindt ('86) indicated that *Mucor corymbifer*, *M. pusillus*, and *Rhizopus Cohnii* were pathogenic for certain animals. However, none of the species of *Mucor* and *Aspergillus* when injected into the blood system of animals gave rise to fructifications in the tissues. Lichtheim pointed out that the negative results of other investigators were to be expected, since all species of *Mucor* and *Aspergillus* are not pathogenic. His predecessors had experimented with diverse species, impure cultures, or with fungi inexactly determined.

Birds are very susceptible to aspergillosis and succumb regularly after a time varying with the quantity of spores injected. Rabbits and guinea-pigs are susceptible to a less degree, and these animals can survive when they receive infections of a small quantity of fungous spores. Dogs, cats, and sheep are immune.

More recently Lucet and Costantin ('00) have established the pathogenic nature of *Mucor corymbifer*. Rabbits succumb to the spores of *M. corymbifer* three to twelve days after the injection of the spores, with lesions in the kidneys and mesenteric glands. The same authors affirm the pathogenic properties of *Rhizopus nigricans* (*Rhizomucor parasiticus*). Intravenous and intraperitoneal injections of *R. nigricans* proved fatal to rabbits and guinea-pigs, but subcutaneous injections were ineffective.

The authors above mentioned assert that the intensity of the toxic action of fungi is proportional to the quantity of the fungous spores injected, and that in this manner it differs from that of pathogenic bacteria in which the intensity of the toxic action is independent, to a large extent, of the number of bacteria injected into the animal. The toxic action of fungous spores is apparently not affected by chemical treatment or by heating for a limited time up to the thermal death-point. The fungous spores may germinate, but no multiplication of cells takes place in the tissues; consequently, in experimental mycotic infections there is no secondary generalization. These mycotic colonies are not directly inoculable into other animals, and in order to infect another animal, it is necessary to "produce a new series of spores in contact with the air."

There are a large number of species of *Mucor* and *Aspergillus* of wide distribution. The most common species of *Mucor* is *M. Mucedo* which is non-pathogenic for animals, as is also *Rhizopus nigricans* (*Mucor stolonifer*), a very widely distributed species which is often considered pathogenic. The species of fungi given below have been considered as injurious to animals. The greater number of these species have been misdetermined, whereas the toxic action

of certain other species of fungi is questionable. A description will be given only for the more important and confirmed species.

PHYCOMYCETES

MUCOR

M. cornealis Sacc. in Cavara, Centralbl. f. Bakt. I. 72:23-37. 1914.

This fungus was isolated by Cavara ('14) from a man's eye which had been struck by a piece of dirt. A week after the accident, there developed a ceratomyces mucorinea, an inflammation of the cornea. A culture of the fungus was sent to Saccardo for determination, who found that it was a new (?) species closely related to *Mucor racemosus* and *M. Regnierii*. It produced injurious effects when inoculated into the blood system of rabbits and guinea-pigs, with the occurrence of lesions in the kidneys. Saccardo's description of this fungus agrees entirely with the descriptions for *M. corymbifer*, including the color of mycelium, the maximum and minimum growth temperatures, and the type and size of sporangia, columella, and spores.

M. corymbifer Cohn, in Schroeter, Kryptogamenflora Schlesien 3:205. 1886.

Mycelium at first snow-white, later a dull gray, hyphae penetrating the substratum or aërial and ascending, hyaline; hyphae of sporangia racemose, bearing 1-12 sporangia; sporangia pyriform, varying in size from the smallest, 10-20 μ , to the largest, 70 μ in diameter; columella conical to hemispherical, finally papillate, brown; spores hyaline, elliptical, 3 $\times$ 2 μ , to ovoid, 4 $\times$ 6.5 μ .

This species was first isolated by Lichtheim ('82) in his laboratory, growing with *Rhizopus Cohnii* on a decoction of bread. It was reported by Paltauf ('85) as occurring in the principal organs of a man dead from generalized mycosis; by Huckel in 1885, and Siebenmann in 1889, as associated with *Aspergillus fumigatus* in the external auditory canal; also in two cases of pulmonary mycosis, by Fürbringer ('76). It has an injurious action on rabbits and guinea-pigs, accord-

ing to Lichtheim ('82), and Berthelat ('03). This species is considered by Lucet and Costantin, ('01) as including several varieties known as *M. ramosus* Lindt, *M. Truchisi* Lucet & Cost. and *M. Regnieri* L. & C.

M. Mucedo L. Sp. Pl. 2:1655. 1764.

Mucor Mucedo is found, in general, on all organic substances of vegetable and animal origin in the process of decomposition, and more particularly on the excrement of animals. It is extremely common in the state of a saprophyte, and has been reported at various times as occurring on man and other animals; in mycosis of man by Hiller in 1874 (cited by Plaut, '03) and Fürbringer ('76). In these observations the determination of the fungus was not sufficiently established, and the demonstrated virulence on animals was possibly due to impure cultures, since Berthelat ('03) finds that it is without action on rabbits and guinea-pigs.

M. Regnieri Lucet & Cost. Archiv. d. Par. 4: 366–384. 1901.

This fungus was isolated from an epidermal lesion on a horse affected with *Oospora (Trichophyton)*, and according to Costantin ('01), was non-pathogenic for rabbits. This organism evidently had nothing to do with the diseased tissue from which it was obtained. Costantin later regarded this fungus as identical with, or as a variety of, *M. corymbifer*.

M. Truchisi Lucet & Cost. Archiv. d. Par. 4:366–384. 1901.

A culture of this species of *Mucor*, obtained by Lucet ('01) from an epidermal lesion on a horse affected with *Oospora (Trichophyton) minimum*, was toxic for rabbits. It is possible that this fungus played no part in the observed affection, and more recent studies of this organism led Costantin to consider it as identical with *M. corymbifer*.

M. (Rhizomucor) parasiticus (Lucet & Cost.) Sacc. & Syd. in Sacc. Syll. Fung. 16:385. 1902.

Rhizomucor parasiticus Lucet et Cost. Rev. Gén. Bot. 12:92. 1900.

This species was observed by Lucet and Costantin ('00) in a woman affected with pseudo-pulmonary tuberculosis. Cul-

tures were made of this fungus and it was found to be a new (?) species which they called *Rhizomucor parasiticus*. Lucet and Costantin in their description of this particular fungus, assert that it varies from *Rhizopus nigricans* only in having branched sporangiophores. This character alone is not sufficient to establish a new genus, since branched conidiophores occur in many species of *Mucor*. The writer has observed branched conidiophores in cultures of *Rhizopus nigricans*. Lucet and Costantin evidently obtained this latter fungus as a contamination of their culture medium, since they examined only the sputum of the diseased person.

M. pusillus Lindt, Archiv f. exp. Path. **21**:269–298. 1886.

This species of *Mucor* was isolated simultaneously with *M. corymbifer* (*M. ramosus* Lindt) by Lindt ('86). The spores produced injurious effects when injected into rabbits. The same species was reported by Jakowski ('89) in a case of otomycosis, but this determination is considered doubtful.

M. racemosus Fres. Beitr. z. Myk. **12**. 1850.

This species is very frequent on decaying organic substances, such as vegetable debris, meat, and insect cadavers. The fungus found by Bollinger, in 1880, in fifteen instances of mycoses in birds, and determined as *M. conoides* or *M. racemosus*, is probably identical with *Aspergillus fumigatus*. According to Berthelat ('03), it is without action on rabbits and guinea-pigs.

M. ramosus Lindt, Archiv f. exp. Path. **21**:275–284. 1886.

Mucor ramosus Lindt is very closely related to *M. corymbifer*, and differs (?) from it only slightly in the size and shape of the spores. According to Lindt ('86), it is toxic for rabbits.

M. septatus Bezold, in Siebenmann, Schimmelmcykosen d. Ohres **97**. 1889.

Rhizomucor septatus Lucet et Cost. Rev. Gén. Bot. **12**: 81–98. 1900.

Mucor septatus was discovered by Siebenmann ('89) in the external auditory canal, evidently as a saprophyte. The description given for this species is incomplete, and no cul-

tures were made. Fisher regards this fungus as identical with *M. racemosus*, which is non-injurious to animals.

RHIZOPUS

R. niger Ciaglinski & Hewelke, Zeitschr. f. klin. Med. 22:626-632. 1893.

This species, isolated by Ciaglinski (cited by Guéguen, '08) from a case of "black-tongue" and determined as *Mucor niger*, had an optimum growth temperature of 25-27°C., and was non-pathogenic for animals. The incomplete description does not permit of an exact determination, and it is possible that Ciaglinski was dealing with *R. nigricans*.

R. Cohnii Berl. & De Toni, in Sacc. Syll. Fung. 7:213. 1888.

According to Lichtheim ('82), this fungus, found with *Aspergillus fumigatus* on bread, has an injurious effect on rabbits. Its occurrence has not been reported in conditions of mycosis.

MORTIERELLA

Neumann mentions an interesting case of the parasitism of a species of *Mortierella* (?) in a cat which had died by asphyxia, presumably as a result of a fungous growth in the trachea. Costantin ('92) considered the fungus a new (?) species, probably belonging to *Mortierella*, since the spores of the known species of *Mortierella* do not germinate at blood temperature, 37°C. This determination cannot be accepted, since it depends exclusively on the presence of echinulate spores.

ASCOMYCETES

ASPERGILLUS

A. aviarius Peck, N. Y. State Mus., Ann. Rept. 44:137. 1891.

This fungus was found by Peck ('91) in the body of a canary that had died after being sick a few days. No culture was made, and according to Wehmer, it is the same as *A. fumigatus*, for the determination was made from old fungous elements.

A. bronchialis Blumentritt, Ber. d. deut. bot. Ges. 19:442-446. 1901.

A species of *Aspergillus* was discovered by H. Chiari, in the trachea of a diabetic patient. Blumentritt ('01, '05) gives a full account of this fungus which agrees with the description of Wehmer for *A. fumigatus*. Blumentritt admits that *A. bronchialis* is very closely related to *A. fumigatus* and can be distinguished from it only by a few minor physiological characters.

A. candidus Link, Observationes 1:65. 1809.

A. candidus was obtained from a patient affected with otitis, in which case it appeared as a saprophyte, its optimum growth temperature being 25°C.

A. flavus Link, Observationes 1:14. 1809.

In pure cultures on various media usually of a yellow-green to a light brownish green color; mycelium sterile, always grayish white and even hyaline; conidiophores not very conspicuous, usually 500-700 μ in length, 7-10 μ thick, the terminal swelling colorless, spherical to clavate, 30-40 μ in diameter, with conidia about 85 μ ; sterigmata undivided, 20 $\times$ 6 μ , crowded, arranged radially; conidia varying in size, spherical, smooth, rarely papillate, 5-7 μ in diameter.

This species, frequently found in the ear as a saprophyte, and *A. flavescens* Wreden, are considered by Siebenmann ('89) as identical. According to Ribbet, the spores of this fungus are toxic for rabbits. Wreden (cited by Plaut, '03) considered *A. flavescens* as a variety of *A. glaucus*, but new descriptions of the former species are lacking, and in recent literature we find *A. flavescens* appearing as a synonym of *A. flavus*.

A. fumigatus Fres. Beitr. z. Myk. 81. 1850.

A. nigrescens Robin, Veg. Par. 518-528. 1853.

Forms a greenish layer in fruiting cultures; mycelium much branched, 2-3 μ in diameter; conidiophores scarcely different from the hyphae, formed in dense tufts, 100-300 μ long, 5-6 μ thick; terminal swelling small, green, clavate, ta-

pering gradually from the base, 10–20 μ thick, with conidia 30–40 μ thick; sterigmata 6–15 μ long; conidia formed in chains, vertically arranged, not radially, spherical, rarely oval, 2–3 μ in diameter, at first hyaline, then gradually changing from yellow to green and finally to brown.

This species has been reported in most cases of mycosis as being very frequently found in the respiratory tract of birds. It affects the eye, ear, and other parts of the body if they are accidentally injured or become diseased, as well as the lungs of men who feed birds in the manner previously mentioned. It occurs in cases of ceratomycesis, according to Leber ('82) and Uhthoff ('83), and in otomycosis reported by Siebenmann ('89). Siebenmann considers *A. nigrescens* Robin as identical with *A. fumigatus*. The species is very toxic for rabbits, guinea-pigs, birds, and monkeys. Dogs and cats, however, are not affected.

A. fontoyonti Guéguen, Compt. rend. Soc. Biol. 66:1052. 1909.

Under the name of "nodosités juxta-articulaires," Jansselme has described a disease which occurs in Indo-China and Madagascar. Two of the cultures which were made closely resemble *A. Tokelau* in growth characters. The description, as given by Guéguen ('09), is that of a slow-growing *Aspergillus*, producing meager fructifications of a greenish white color after a growth of three weeks on Raulin gelatin medium. No liquefaction of gelatin takes place before fourteen days, and only a slight liquefaction after a month. Its optimum growth temperature is 22–25°C., but no development takes place at 37°C. From these observations it is difficult to understand why an injection of spores of this fungus should prove fatal to rabbits and guinea-pigs.

A. glaucus Link, Observationes 1:67. 1809.

This species has been reported as the green mould occurring frequently in the air sacs of birds, but more precise observations tend to show that these parasites are mostly *A. fumigatus*.

A. malignus Lindt, Archiv f. exp. Path. **25**:257-271. 1889.

Spores of this fungus, found by Lindt in a case of otitis, when introduced into animals, produced death in a few days after injection. However, according to Wehmer ('98), *A. malignus* Lindt is evidently *A. fumigatus* Fres.

A. nigricans Cooke, Jour. Quekett Micr. Club II. **2**:140. 1885.

This species, according to Siebenmann, is *A. fumigatus* and *A. nigricans* Wreden is *Sterigmatocystis nigra* Van Tieg. Under the name of *Otomyces purpureus* Wreden describes what he considered as the ascospore form of *A. nigricans*; but according to Gedoelst ('02) these forms of development were nothing more than pseudo-perithecia of *Sterigmatocystis nidulans*. Wehmer ('98), however, considers *A. nigricans* Cooke only as *Sterigmatocystis nigra* Van Tieg.

A. repens (Corda) Sacc. in Michelia Commentarium Mycologicum **2**:577. 1882.

This species was reported by Siebenmann ('89) as having been present at three different times as a saprophyte in the ear. It is more likely that Siebenmann was dealing with *A. glaucus* which differs but slightly from the descriptions given for *A. repens*.

A. Tokelau Wehmer, Centralbl. f. Bakt. I. **35**:140-146. 1904.

Mycelium hyaline, very delicate, 1-2 μ thick, branched, growing between the epidermal elements, septate; conidiophores usually small, 100 μ , sometimes 500-900 μ , long, with a diameter varying from 8-12 μ in the smallest to 30 μ in the largest, hyaline, smooth; terminations light brown to yellow; pedicel simple, rarely irregularly branched, hyaline, smooth, thin-walled, 5-13 μ wide; sterigmata undivided, flask-shaped, more or less numerous, 5-9 $\times$ 2-3 μ , usually arranged radially; conidia globose, rarely globose to ellipsoidal, echinulate, isolated or only in short chains, size varying from 3-12 μ in diameter.

The Samoa disease or tokelau, a skin disease occurring in certain Oceanic islands of the South Sea—Fiji, Gilbert, and

Solomon Islands—was described by Patrick Manson as “*tinea imbricata*.” In the characteristic lesions Tribondeau found spore-bearing organs similar to the conidiophores of *Aspergillus*, but he was not sure of this determination and therefore called the fungus “*Lepidophyton*.” This, however, did not clear up the situation. Fortunately, Wehmer ('04) obtained a culture of this organism and found it to be a new species of *Aspergillus*. The fungus develops in the epidermal tissues of the body and extremities. The lesions have much the appearance of ringworm and also occur in superficial cancer ulcerations.

STERIGMATOCYSTIS

S. nigra Van Tieg. Soc. Bot. Fr., Bul. 24:102. 1877.

Evidently a species easily recognized by the dark brown color of the conidial masses; conidiophores very crowded, 2 mm. high; pedicel about 18μ in diameter, with walls 2μ thick, hyaline; terminations globose to subglobose, 80μ in diameter, with conidia 130μ in diameter; numerous sterigmata on all sides, radially arranged, very slender, branched; primary sterigmata clavate, $26 \times 4.5\mu$; secondary sterigmata $8 \times 3\mu$; conidia in long chains, small, spherical, smooth, or echinulate in old cultures, $2.5\text{--}4.5\mu$, violet-brown; hyphae 3μ in diameter.

This species was cited for the first time as a parasite by Cramer, while soon after Fürbringer ('76) reported the same fungus occurring in the lungs of a man. Wreden describes a mould frequent in otomycosis which he termed *Aspergillus nigricans*, but according to Siebenmann ('89) it is *Sterigmatocystis nigra*, probably the same fungus that Costantin and Lucet ('03) reported under the name of *S. pseudonigra*. Lucet, Costantin, and others have found that *S. nigra* is non-pathogenic for animals.

SACCHAROMYCES

S. anginea Troisier & Achalme, Archiv. d. Méd. Exp. et d'Anat. Path. 5:29-37. 1893.

The first observation of a parasitic yeast in man is that of Troisier and Achalme ('93) in a patient with a condition

clinically resembling thrush. A microscopic examination of substances taken from the pharynx revealed the presence of ovoid cells $8-9 \times 5-6\mu$, united in groups of eight or ten. Budding took place only at the extremities of the cells. In culture asci with four globose ascospores, 2μ in diameter, were formed. There was no liquefaction of gelatin and no film formation in liquid nutrient solutions. Saccharose present in quantities less than 10 per cent in nutrient solutions was completely changed to alcohol.

S. granulatus Vuillemin & Legrain, Archiv. d. Par. 3:237-268. 1900.

This yeast was found by Vuillemin and Legrain in a tumor of the inferior maxillary region of a man. It appears in the form of oval or elliptical cells $2-10 \times 3-4\mu$, with a membrane covered with isolated granulations or striations disposed in regular lines. The cells form one, rarely two, buds and enclose fat bodies of a red color. Chlamydospores are sometimes present in cultures. In liquid media the fungus does not produce a film but forms a red sediment. It does not liquefy gelatin. Two to four spherical or elliptical spores appear in each ascus. This yeast is slightly pathogenic for the rabbit when inoculated intraperitoneally.

SACCHAROMYCOPSIS

S. guttulatus (Robin) Schiönning, in Robin, Veg. Par. 327-331. 1853.

This species was discovered by Remack and Robin, and later studied by Casagrandi and Wilhelmi. It is normally present in the stomach and intestines of the rabbit, and of certain birds and reptiles.

The cells of this fungus are very large, $6-16 \times 2-4\mu$, oval or more or less rectangular, and united in groups of two or three. Budding takes place at the two poles of the cells. The optimum growth temperature is $35-37^{\circ}\text{C}$. No film is observed in liquid media. Ascospores, one to four in each ascus, are present only in the excrement of the rabbit. The spores are oval with a double membrane. At the beginning of germination, the exospore breaks at one end or at the side of the

ascospore, and then growth takes place by budding. This yeast ferments dextrose and inverts saccharose. According to Casagrandi, it is pathogenic for the guinea-pig and rabbit by subcutaneous injections.

ENDOMYCES

E. albicans Vuillemin, Compt. rend. Acad. Paris 127:630-633. 1898.

The organism producing thrush has been variously classified as *Sporotrichum* Gruby (1842), *Aphthophite* Gruby (1844), *Oidium* Robin (1853), *Stemphylium* Hallier (1866), *Syngospora* Quinquaud (1868), *Mycoderma* Grawitz (1877), *Saccharomyces* Reess (1877), *Monilia* Plaut (1888), *Dematium* Laurent (1890), and *Empusa* Henri (1896). In its parasitic life *E. albicans* develops a more or less extended membranous layer, at first white, then gray, on the mucus of the primary digestive passages—mouth, pharynx, and oesophagus. This membrane, which attains a thickness of 1-2 mm., does not adhere to the mucus and is easily detached. A microscopic examination shows it to be made up of filaments, usually from $3-5 \times 50\mu$. The terminal cells may often attain a length up to 600μ . According to Linossier and Roux ('99), this organism does not grow in saliva. This peculiarity accounts for the fact that thrush occurs only in infants, more frequently during the first few months of life when the salivary secretion has been insufficiently established, and, in general, in all cases of infection accompanying a diminution of the secretion of saliva.

On different media the fungus develops either by budding, like a yeast, or by the elongation and division of cells, as in *Monilia*. On carrot the mycelium is very well developed, whereas in nutrient liquids only yeast cells are present. According to Vuillemin, the filamentous form is the normal method of vegetation, the yeast cells appearing in conditions of malnutrition. In sugar solutions at a temperature of 30-35°C., chlamydospores form at the end of the filaments.

The asci were discovered by Vuillemin ('98) in old cultures on beet. They appear as large, ovoid or elliptical asci, $4-5\mu$ in diameter, formed by a lateral or terminal bud-

ding of the mycelium or derived by the germination of chlamydospores. The asci contain four ascospores, formed in a manner similar to those of *E. capsularis*, slightly reniform, $2.8-3.5 \times 1.75-2 \times 1.2-1.4\mu$, with a thick membrane. The germination of these ascospores has not been observed.

Development takes place at a temperature of 20–39°C. on slightly acid, solid, or liquid media. In sugar solutions and fruit juices growth takes place slowly, with the formation of a flocculent deposit but with no film on the surface. The fungus coagulates milk after 20–30 days and ferments dextrose slightly. Certain authors believe that there exist many varieties of *E. albicans*, only some of which have the function of producing spores.

Castellani ('11) isolated from cases of bronchomycosis in Ceylon, twenty-two strains of *Endomyces* which, in microscopic appearance and cultural characters, closely resembled *E. albicans*. Fourteen strains were identical and corresponded to *E. tropicalis*, whereas the other eight strains differed from *E. tropicalis*, and from each other. The behavior of the different strains, especially toward sugar solutions, indicated that there are nine different species. Castellani believed that six of these species were parasitic, but he was not certain about the other three. It may be possible that a similar condition as to the plurality of species of *Endomyces* affecting man also exists in the temperate zone.

Before describing the better-known pathogenic yeast-like fungi, it may be well to consider first the development of the parasitic theory of cancer. This disease, more than any other human ailment, has been a fruitful field for the discovery of such forms as resemble yeasts.

The one common characteristic of cancers is the power of cell proliferation, and the problem that many scientists to-day are undertaking is the causes underlying such proliferation. Many investigators take the view that cancer has some specific and demonstrable cause.

By the continued division of the carcinoma cells, masses of tissue are formed which grow out into lymph channels. Mechanically obstructing the normal activities of surround-

ing tissues, or breaking through such tissues, they give off small groups of free cells which may be carried by the blood to various parts of the body, there to set up independent growths (metastases). With the local disturbances caused by such abnormal growths, many normal cells are killed, whereas the cancer cells themselves undergo hyperplasia and hypertrophy. The progress of cancer is accompanied by the degenerating of various kinds of cells, and these different structures are the things which have been interpreted by various investigators as x-bodies, amoebae, coccidia, protozoa, or other organisms.

Many of the structures thus interpreted as organisms are characterized by capsules which some investigators have interpreted as parts of an invading organism. Cell invasions are common in cancer tissue, but the capsules are only condensations of the invaded protoplasm. Pianezze ('96) observed similar bodies in the nuclei of cancer cells, and interpreted both these and the cytoplasmic forms as colloidal degenerations of the chromatin and cytoplasm.

Although these cell inclusions in human cancer cannot be considered as organisms, it does not follow that real organisms are not present. Later stages of the disease are particularly suitable for secondary infection, and exposed surface lesions form a suitable medium for the growth of bacteria, yeast-like organisms, and other fungi.

San Felice ('95) made a series of inoculation experiments on animals with yeast-fungi obtained from various sources, principally from the juice of fruits. During the course of these experiments he found one species, *Torula neoformans*, which, if inoculated into animals, produced the formation of neoplasm. The same author ('96) also discovered *Cryptococcus lithogenes* in a lymphatic ganglion of a cow that died as a result of a primary carcinoma of the liver. From this observation he seemingly obtained the confirmation of his ideas on the pathogenic rôle of cryptococci in the formation of malignant tumors. His parasitic theory of cancer was based on the presence of cryptococci in tumors, the isolation of cryptococci

from diseased tissues, and the results of inoculation experiments on animals.

The presence of cryptococci in tumors has been mentioned by a large number of authors, but the tumors thus parasitic do not all pertain to the type of malignant tumors. Binaghi ('96) investigated fifty-three cases of epithelioma and isolated parasitic organisms in forty instances. The failure to obtain parasites in the remaining thirteen, according to this author, may be due to the fact that the part examined was either in an early stage of development or not infected. These organisms, identical with cryptococci in their morphological and physiological characters, were not found in other pathological or normal tissue. Consequently, they were considered as the specific cause of epithelioma. Maffucci and Sirleo ('98) obtained ten or more cultures of yeasts from the thirty-nine tumors which they examined. Only one was pathogenic for guinea-pigs, and in these animals it produced fibrinous pneumonitis and abscesses under the skin or in the kidneys. The results indicated that a new formation of sarcoma tissue did not take place. Roncali, Corselli, and Plimmer obtained cultures of fungi from malignant tumors, but only in a few cases were they pathogenic for animals. Leopold ('00) reported having isolated pure cultures of cryptococci from over 80 per cent of the non-ulcerated tumors investigated, the cultures having been obtained from the center of the diseased tissue.

Loeb, Moore, and Fleisher ('13) were unable to confirm the results of Leopold. Of the seventeen tumors examined, only one gave a culture of a yeast-like organism. In no case of human cancer has the causative significance of a microörganism so far been proved. Moreover, Busse ('03) was never able to obtain a single culture of *Cryptococcus* from non-ulcerated tumors.

No one has been able to demonstrate the development of tumors histologically comparable to cases of sarcoma, by the inoculation experiments on animals with cultures of *Cryptococcus*. By inoculation of *C. tumefaciens* in the white rat, Curtis obtained results in which "la tumeur etait iden-

tique a celle de l'homme, c'est-a-dire constituée par une infiltration du parasite dans les mailles du tissu cellulaires." This, however, does not imply that Curtis obtained a sarcoma-like tumor.

FUNGI IMPERFECTI

CRYPTOCOCCUS

The species of *Cryptococcus* here given were reported as having been obtained from tumors, but their rôle in the production of these malformations has not been determined.

C. Corsellii (Corselli & Frisco) Neveu-Lemaire, in Corselli & Frisco, Centralbl. f. Bakt. I. 18:368-373. 1895.

This species was isolated by Corselli and Frisco ('95) from a sarcoma of the mesenteric ganglia of a man. This fungus has dark cells, globose, and of variable dimensions. It is easily cultivated on gelatin, agar, and bouillon, neutral or alkaline. It can give rise to a slight fermentation and is pathogenic for guinea-pigs, dogs, and rabbits, by intraperitoneal inoculations.

C. degenerans Vuillemin, in Roncali, Centralbl. f. Bakt. I. 18:353-368. 1895.

Roncali ('95) observed this species in the ganglion of the armpit of a woman affected with cancer of the breast. In the cancer the cells are globose, rarely oval or reniform, isolated or in groups. In cultures the cells are globose or elliptical. On sugar nutrient liquids this fungus produces a film. On gelatin the colonies are irregular in outline and of a light yellow color. Gelatin is not liquefied and saccharose is not fermented. It is pathogenic for the guinea-pig by intraperitoneal injections. The autopsy revealed an abscess of the mesenteric ganglia, which was produced by degeneration products; the normal cells were rarely found in these lesions.

Cryptococcus of Gotti & Brazzola, in Guilliermond, Les Levures. 1913.

This species was found by Gotti and Brazzola in a myxosarcoma of the nasal fossa of a horse. The cells are

of variable dimensions, round or oval, with granular contents, enveloped by a membrane of double contour and a mucilaginous capsule that is often stratified. On gelatin or agar, the colonies are white with denticulate margins. Acid gelatin is liquefied. It is pathogenic for the guinea-pig but not for other animals.

C. farciminosus Rivolta & Micellone, in Fermi & Aruch, Centralbl. f. Bakt. I. 17:593-600. 1895.

This species has been considered as the parasite of "African glanders." The cells are globose or oval, sometimes acuminate at the two poles. It grows with difficulty on all media. Fermi and Aruch have described the globules, which they considered ascospores, in the cells of this yeast.

C. Gilchristi Vuillemin, in Gedoelst, Les Champ. Par. 1902.

This fungus was obtained by Gilchrist in a case of scrofulodermitis. It is only slightly pathogenic for animals.

C. granulomatogenes (San Felice) Vuillemin, in San Felice, Zeitschr. f. Hyg. 44:364-396. 1903.

This fungus was discovered by San Felice in the lung nodules of a hog. It is only slightly pathogenic for animals.

C. hominis (Busse) Vuillemin, in Busse, Die Sprosspilze, in Kolle & Wasserman, Handbuch 1:631-700. 1903.

This species was discovered by Busse ('03) in periosteal lesions of the tibia of a woman. *In situ* the oval or globular cells are united in variable numbers in a substance of a homogeneous aspect, constituting a sort of common capsule. This homogeneous substance is not present in cultures. The cells have a double-contoured membrane that becomes thicker with the age of the culture.

It is easily cultivated on all media between 15 and 38°C. In liquid media it forms a deposit of yeast cells and a thin film on the surface. It does not liquefy gelatin. On potato the colonies rapidly unite to form a thick white layer. The fungus ferments dextrose and is pathogenic for rabbits, white mice, and dogs.

C. linguae-pilosae (Raynaud & Lucet) Vuillemin, Lucet, in Archiv. d. Par. 4:262-287. 1901.

This fungus was discovered by Raynaud and Lucet in the disease of "black-tongue." Lucet ('01) in his experimental studies demonstrated that this fungus did not reproduce the disease. According to Guéguen and Thaon, this yeast acts only when associated with *Oospora lingualis*. There is, then, in these two fungi a sort of symbiotic relation.

This organism has spherical, ovoid, or elliptical cells, $4-17 \times 6\mu$. It grows on most media and in liquids forms a white film after ten hours at 37°C . It ferments glucose and levulose, with the production of alcohol and carbon dioxide. Development is accompanied by the production of acid, but no liquefaction takes place on gelatin. It has a slight pathogenic action on certain animals and is without effect on guinea-pigs and rabbits by subcutaneous or intraperitoneal inoculations.

C. lithogenes (San Felice) Vuillemin, in San Felice, Zeitschr. f. Hyg. **21**:32-58, 394-420. 1895-96.

San Felice isolated this parasite from the lymphatic ganglia of a cow that died as a result of primary carcinoma of the liver, with extensions of the infection to the entire lymphatic system. In the tissues the cells are more or less spherical, of variable dimensions, enclosed by a refringent membrane. This species grows well on all media. On glucose bouillon it forms an abundant deposit of yeast cells and often a film on the surface. It does not liquefy gelatin. It forms white yeast-like colonies on agar, and on potato the colonies become dark brown in color. It is pathogenic for the guinea-pig, mouse, and sheep.

C. niger (Maffucci & Sirleo) Vuillemin, in Maffucci & Sirleo, Zeitschr. f. Hyg. **27**:1-30. 1898.

This species was discovered by Maffucci and Sirleo ('98) in a pulmonary lesion of a guinea-pig inoculated with the liver of an embryo taken from a tubercular mother. It is pathogenic for animals only after a long time. The cultures sterilized by heat are toxic for guinea-pigs.

C. Tokishigei Vuillemin, in Tokishige, Centralbl. f. Bakt. **I**. **19**:105-113. 1896.

This fungus is considered by Tokishige ('96) as producing in Japan the disease of horses known as "farcin." It is non-pathogenic for rabbits, guinea-pigs, and dogs, by subcutaneous inoculations. The pathological products of this fungus have more effect on animals than inoculations of the cultures. Inoculation of this organism in the horse produces lesions after varying lengths of time, but without causing the death of the animal.

OOSPORA

The longest-known fungous disease of the skin is favus, which previous to 1839 included a large number of different skin affections. When its contagious nature was discovered by Schonlein, investigators began searching for the relation of fungi to skin diseases. Heusinger in 1826, and Remak in 1837 (both cited by Virchow, '56) observed mould-like filaments in the scales of tinea. The latter author in 1847 succeeded in growing cultures on apple, again producing the characteristic lesions, presumably by inoculating his arm with these cultures. Gruby ('43) independently discovered three different fungi associated with as many types of ringworm. Two of these fungi were *Oospora*, and the other was *Sporotrichum* (*Microsporon*) *Audouini*. His description of these fungi was very good, yet no one at that time suspected that, under the name of *Porrigio decalvans*, he was describing fungi which were the cause of ringworm. Very little attention was given to these discoveries until Sabouraud began his investigations in 1892, which differentiated the many varieties of *Oospora* (*Trichophyton*) and *Sporotrichum* (*Microsporon*).

Oospora is often associated with mycosis of the lung and with *Endomyces albicans* in the mouth. *Oospora pulmonalis* brings about a degeneration on the surface of the trachea and walls of the bronchia, where white granules are found similar to those caused by *Actinomyces*. The alveolar structure is filled with mycelium, and in certain places real abscesses are found constituted by degenerated tissue filled with mycelial debris. In lesions of the mouth, Roger, Bory,

and Sartory ('09) discovered *Oospora buccalis* associated with *Endomyces albicans*. In other diseases caused by *Oospora*, the fungus may occur as a saprophyte or as a parasite. These fungi are very closely related to each other and cannot always be differentiated sharply.

Oospora of *Trichophyton*.

Gruby ('43) was the first to give a clear description of the *Oospora* parasite that causes epidemic and endemic diseases of the skin. The morphology of these fungi has been studied in the lesions which they produce and in cultures on artificial media. This fungus develops into filaments composed of short segments. The dimensions of the cells in the same filament are approximately equal. Their diameter remains invariably the same for the same species, but the length of the cells may vary 1 to 2 μ . The cells may be spherical or oval, the mycelial filament being moniliform and easily dissociated into its different elements. When the mycelial cells are cubical, the filaments are more or less extended and not easily dissociated.

The classification of the varieties affecting man, according to the infection, falls into two groups. The endothrix group contains the varieties of human origin which develop in the hair between the cuticle cells and grow exclusively within the hair structure. The ectoendothrix group of parasites, probably of animal origin, develops in the hair and proliferates in the follicle outside.

More often the scheme of Sabouraud is used, in which are considered the mode of infection and principally the cultural characters of the isolated fungus when grown on "proof agar." There are over thirty varieties of *Oospora* of *Trichophyton*, and their differences are chiefly cultural.

Matruchot and Dassonville ('99), reasoning from analogies, place the parasites of *Trichophyton* in the *Ascomycetes*, in the family *Gymnoascaceae*. The asexual type of development in the *Gymnoascaceae*, according to these investigators, can serve to characterize these fungi with the same degree of precision as the complete forms. The demonstrated affinity

of *Oospora tonsurans* with *Ctenomyces* rests on the similarity of characters in the conidial stage of development. In old cultures of *O. tonsurans* may be found multicellular chlamydospores, spindle-spores, and serrate septate hyphae. This fungus, according to Matruchot and Dassonville, is an imperfect form of a species of *Ctenomyces*, still unknown, which has adopted a parasitic mode of development and consequently has lost the faculty of producing perithecia and ascospores. The serrate spiral hyphae, present in cultures of *O. tonsurans*, are considered as traces of asci formation.

O. tonsurans (Malmsten) Sacc. & Trav. in Sacc. Syll. Fung. 20:236. 1911.

The mycelium of this fungus usually fills the entire hair without having passed through the cuticle. The filaments are more often simple, rarely dichotomously branched. The fungus is made up of squarish cells 4–5 μ long, arranged in chains that follow the direction of the hair.

O. porriginis (Mont. & Berk.) Sacc. Syll. Fung. 4:15. 1886.

Oidium porriginis Robin, Veg. Par. 477–488. 1853.

Gruby ('43) demonstrated that the fungus parasite which he had independently found in favic lesions, was the cause of the affection, and in the following year made successful inoculations with this fungus in the human skin and the skin of animals. In 1845 Remak separated the fungus from the genus *Oidium* to which it had been assigned, and created the genus *Achorion*, with the name *Achorion Schönleini* for this specific fungus. The characteristic lesion is a small yellow disc with a cup-like depression in the center. Both in color and in shape, it resembles a honeycomb, hence the name which comes from the Arabic *Sahafts*, meaning honeycomb. In the middle ages the disease was called tinea, which name is still retained. Matruchot and Dassonville ('99) also consider *O. porriginis* as belonging to the family *Gymnoascaceae*, but this classification cannot be regarded seriously.

SPOROTRICHUM

S. (Microsporon) Audouini Gruby, Compt. rend. Acad. Paris 17:301–303. 1843.

Sixty to 65 per cent of the fungous affections of the scalp are caused by *Sporotrichum* or *Microsporon*, including eleven varieties which are divided by Sabouraud into two groups: those of the *Sporotrichum Audouini* type which give slow-growing cultures on artificial media, and those of animal origin which yield rapidly growing cultures. The disease, frequently derived from domestic animals, rarely attacks the glabrous skin, and contagion is more frequent from case to case. At least four varieties of *Sporotrichum* are parasites common to man and animals.

Upon microscopic examination of the diseased hair, the fungus appears in crowded cells, 2–3 μ in diameter, irregularly arranged so as to form a continuous covering of the hair without penetrating the cuticle. In the interior we find delicate parallel filaments of large cells. In infection with *Microsporon* the growth of the fungus progresses from the tip of the hair to the lower parts.

S. Furfur (Robin) Sacc. Syll. Fung. 4:100. 1886.

An affection of the cuticle, called “tinea versicolor,” is characterized by the yellowish or brownish discoloration of the lesions, which at one time were classed with the group of pigmentary stains. The color of the lesion is subject to great variation, not merely in different patients, but in different regions of the same patient. This affection caused by *S. Furfur* was first discovered by Eichstedt in 1846. Little is known of the mycological characters of this parasite.

S. (Microsporon) minutissimum Sacc. in Gedoelst, Les Champ. Par. 1902.

An epidermomycosis, erythrasma, presenting some points of resemblance to tinea versicolor, is characterized by brownish scaly patches which appear usually in the genitocrural region. The elements of this species are very small, and in preparations, appear as spores and threads of mycelia arranged almost in the same manner as the elements of *S. Furfur*.

The recognition of sporotrichial infection other than those occurring in skin diseases, is of recent date. The infection,

in a case described by Schenk in 1898, started in the index finger and led to the formation of a series of subcutaneous abscesses connected by a chain of chronic lymphangitis along the arm. This new parasitic fungus was obtained by cultures from the lesions. In 1906 Beurmann (cited by Pinoy, '11) again called attention to this parasite occurring in multiple, widely distributed, gummatous lesions. *Sporotrichum* affects not only the skin and subcutaneous tissue, but also the mucous membrane. Intramuscular and periosteal gummas, and even pulmonary abscesses may be caused by this fungus.

GLENOSPORA

G. Graphii (Siebenmann) Vuillemin, Compt. rend. Acad. Paris 154:141-143. 1912.

Verticillium Graphii Harz & Bezold, in Siebenmann, Die Schimmelmücken d. Ohres 95. 1889.

This fungus has been reported by Hassenstein, Steudener, Bezold, and Siebenmann as occurring in otomycosis. In seven cases of otomycosis *Verticillium* has been incriminated four times. This botanical classification of the fungus was not certain, and has not been verified in later investigations. Siebenmann considers Steudener's *Trichothecium*, Hallier's *Stemphylium*, and Harz and Bezold's *Verticillium*, as identical organisms. Vuillemin ('12) followed the development of the above fungus in culture and was able to explain the divergent opinions as to its identity. No degree of regularity in form and position is attained by the conidia, which are dark, one-celled, and irregularly inserted on the mycelium like the conidia of *Glenospora* Berk. & Curt. This then eliminates as parasites of man the genera *Stemphylium*, *Cephalothecium*, *Verticillium*, and the pseudo-genus *Graphium*.

The species of fungi mentioned above may be summarized in the following manner:

PHYCOMYCETES

MUCOR

M. cornealis Sacc. is *M. corymbifer* Cohn.

M. corymbifer Cohn has proven toxic for rabbits and

guinea-pigs. It occurs frequently as a saprophyte.

The occurrence of *M. Mucedo* L. in instances of mycosis is doubtful; it is non-pathogenic for animals.

M. Regnieri Lucet & Cost. is *M. corymbifer* Cohn.

M. Truchisi Lucet & Cost. is *M. corymbifer* Cohn.

M. parasiticus Lucet & Cost. (Sacc. & Syd.) is *Rhizopus nigricans*.

M. pusillus Lindt has not been reported in cases of mycosis.

The occurrence of *M. racemosus* Fres. in cases of mycosis is doubtful. It is non-pathogenic for animals, and those instances of mycosis in which *M. racemosus* Fres. were reported are probably due to *Aspergillus fumigatus* Fres.

M. ramosus Lindt is *M. corymbifer* Cohn.

M. septatus Bezold, as reported in cases of mycosis, is *M. racemosus* Fres.

RHIZOPUS

R. niger Ciaglini & Hewelke is *R. nigricans*.

R. Cohnii Berl. & De Toni has not been reported in cases of mycosis; it is toxic for animals.

MORTIERELLA

The determination of Costantin that a species of *Mortierella* was present in a mycosis of a cat has not been accepted.

ASCOMYCETES

ASPERGILLUS

A. aviarius Peck is *A. fumigatus* Fres.

A. bronchialis Blum. is *A. fumigatus* Fres.

A. candidus Link has been reported as a saprophyte.

A. flavus Link is also a saprophyte.

A. fumigatus Fres. is frequently found in lung mycosis, more particularly in birds. Most cases of mycosis are due to this species of *Aspergillus*.

A. fontoyonti Guég. is a parasitic fungus that produces epidermal lesions. It is toxic for animals.

Fungi reported under the name of *A. glaucus* Link are *A. fumigatus* Fres.

A. malignus Lindt is *A. fumigatus* Fres.

A. nigricans Cooke is *A. fumigatus* Fres.

A. nigricans Wreden is *Sterigmatocystis nigra* Van Tieg.

A. repens (Corda) Sacc. is a saprophyte and the same as *A. glaucus* Link.

A. Tokelau Wehmer is a true parasite.

STERIGMATOCYSTIS

S. nigra Van Tieg. is a saprophyte and non-pathogenic for animals.

SACCHAROMYCES

S. anginae Achalme & Troisier and *S. granulatus* Vuillemin & Legrain are the only two species of *Saccharomyces* reported as animal parasites.

SACCHAROMYCOPSIS

S. guttulatus (Robin) Schiönning is normally a saprophyte.

ENDOMYCES

E. albicans Vuillemin produces the disease known as thrush.

FUNGI IMPERFECTI

CRYPTOCOCCUS

There are a large number of cryptococci that have been described, most of them having been obtained from tumors. Their morphology and botanical classification have not been sufficiently established to permit a satisfactory summary at the present time.

OOSPORA AND SPOROTRICHUM

Certain species of *Oospora* and *Sporotrichum* are producers of skin diseases.

GLENOSPORA

G. Graphii (Siebenmann) Vuillemin is evidently a saprophyte.

EXPERIMENTAL DATA

In a preliminary set of isolations a number of yeast cultures were obtained from certain fruits, the sap of trees, and the seeds of various plants growing in the Missouri

Botanical Garden and vicinity. The finding of yeast-like organisms on the seeds of native plants at once suggested the possibility of obtaining yeasts from foreign sources. Four hundred and ninety-three samples of seeds of indigenous plants were received from botanical gardens of Europe, Asia, Africa, Australia, West Indies, Central America, South America, and the Oceanic Islands. A quantity of each kind of seed was separately placed in sterilized test-tubes containing sterilized distilled water. These test-tubes were set aside for twelve hours and then gelatin plates were made by inoculating tubes of sterile beer-wort gelatin with a mm. platinum loop of the water in which the seed had been standing. Only two yeast-like fungi were obtained from these seeds, although a large number of different moulds and bacterial colonies were isolated. The presence of so few yeast-like fungi may have been due to the dry condition which is unfavorable for the survival of many such organisms. Because so few yeasts were obtained from foreign sources, the investigation was later restricted to the material available in and near St. Louis.

Cultures of yeasts were obtained by plating from beer-wort gelatin inoculated with minute quantities of infected fruit juices. Pieces of various fruits and the basal portions of flowers were placed in test-tubes of sterilized water for about twelve hours. Pieces of fruits were also placed in moist chambers for about three days or more. In either case gelatin plates were made by direct inoculation from the moist material. Holes were made in the trunks of a limited number of trees by means of a sterilized auger, one-eighth inch in diameter and extending a short distance beyond the cambium. After three days, samples of the tree sap that had collected in the cavity were transferred to test-tubes of sterilized distilled water by means of a sterilized platinum wire. Gelatin plates were then made directly from the water suspension after it had been standing for about twelve hours. Approximately 850 different sources were examined for yeasts. In all, 180 different strains of fungi were finally obtained from herbaceous seeds, fruits, fruit juices, sap of

trees, and the nectar of flowers. A second and a third series of plates were made from the original colonies, and in most cases this was sufficient for obtaining pure cultures.

Beer-wort gelatin had many advantages over other media, such as beer-wort agar, glucose agar, and potato agar, which are often used for the isolation of these organisms. On agar the growth of bacterial colonies is more rapid than that of the yeast colonies during the first period of incubation; on gelatin the reverse is true, especially with an increase of acidity and a higher percentage of gelatin. The beer-wort gelatin used in these experiments was made by diluting hopped beer-wort with an equal quantity of distilled water, and then adding 12-15 per cent gelatin; the final reaction of the medium was +5 to +7.

The 180 different yeast fungi finally acquired were tested for growth on blood serum medium at blood temperature. It was impracticable to make inoculation experiments on animals with all of these cultures. The blood serum test, therefore, was made with the purpose of eliminating all the cultures that would not grow at 37°C. and presumably under some of the conditions that are to be met with when an organism is introduced into the blood system of higher animals.

The blood serum medium was made by adding one part of nutrient bouillon, obtained from veal, with 1 per cent dextrose, to 3 parts of ox blood serum. The medium was then sterilized at 60°C. for one hour on five successive days and finally coagulated in an inspissator at 75°C. All the tubes were incubated at 37°C. for twenty-four hours in order to eliminate any that were not sterile. The yeast cultures were then transferred to the blood serum medium and kept at 37°C. in a moist chamber for ninety-six hours. The growth appearing after this time was examined microscopically to see if there was any contamination by bacteria. Of the 180 cultures thus tested only twelve strains were obtained which grew under these conditions. These twelve strains included three red, five white, and four black

yeast-like fungi. The sources from which they were derived were as follows:

Culture No. 1. *Torula* sp? from the sap of *Halesia carolina*.

Culture No. 2. *Torula* sp? from the sap of *Pinus sylvestris*.

Culture No. 3. *Torula* sp? from the nectar of *Salvia splendens*.

Culture No. 4. *Torula* sp? from elderberry wine spontaneously fermented.

Culture No. 5. *Torula* sp? from the nectar of *Oenothera* sp?

Culture No. 6. *Torula* sp? from the seeds of *Zea Mays*.

Culture No. 7. *Alternaria* sp? from the fruit of *Ribes Grosularia*.

Culture No. 8. *Alternaria* sp? from the sap of *Pinus austriaca*.

Culture No. 9. *Oospora* sp? from the sap of *Morus alba*.

Culture No. 10. *Alternaria* sp? from the sap of *Populus tremuloides*.

Culture No. 11. *Alternaria* sp? from the seeds of *Sorghum vulgare*.

Culture No. 12. *Alternaria* sp? from the seeds of *Rhus glabra*.

Culture No. 13. A variety of *Saccharomyces cerevisiae* obtained from "Yeast Foam." This culture was used as a control in all the physiological experiments.

Culture 1, Torula sp?—This culture, obtained from the sap of *Halesia carolina*, when grown on beer-wort gelatin and beer-wort agar, develops into spherical colonies of a deep red color, 1–2 mm. in diameter. It may be distinguished from cultures 2 and 3 by its growth on yeast-water agar, on which the colonies attain the size of 2 mm. in diameter—that is, five to ten times the size of the other two

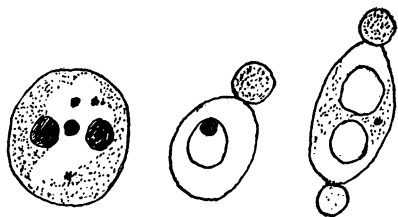


Fig. 1. Culture 1. Cells×2000.

red cultures of *Torula*. It liquefies gelatin rapidly, but culture 2 liquefies gelatin very slowly, and culture 3 not at all. Growth takes place by budding, as in the true yeasts. The cells (fig. 1) are oval or spherical, varying in size from $3-4 \times 4-5\mu$. In sugar nutrient solutions the organism develops rapidly, with the formation of an abundant sediment of yeast cells; but the film, if any, is very thin and made up of colonies loosely connected. In acid yeast-water solution a yellow-brown sediment is formed except in the presence of malic acid in which case the red pigment has practically disappeared, the sediment being almost white. No spores are formed on moist porous plates or on Gorodkowa's test medium, although in many cells there appeared two to four fat globules that are much like endospores in appearance.

Culture 2, Torula sp?—The cells of this strain (fig. 2) vary in size from $3-3.5 \times 4-6\mu$. Growth is by budding from all

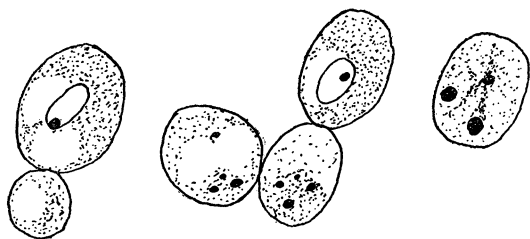


Fig. 2. Culture 2. Cells $\times 2000$.

sides of the mother cell and without the formation of mycelium. Development at 37°C . is more rapid than at room temperature. Cultures grown in yeast water with the addition of saccharose, glucose, levulose, maltose, and lactose, produce a heavy red deposit of yeast cells and a slight ring formation on the surface of the liquid. In yeast water containing 1 per cent of acids—citric, malic, tartaric, and succinic acids—development is as good as in the sugar nutrient solutions. On yeast-water agar the colonies are very small, elliptical or oval in outline, and .2 mm. in diameter. Gelatin is slowly liquefied. No spores are formed.

Culture 3, Torula sp?—This organism, obtained from the nectar of *Salvia splendens*, is brownish red in color. The cells

(figure 3) are oval, $2.8-4 \times 3-6\mu$. In acid yeast-water solution growth is very rapid; and in the presence of lactic and citric acids, only isolated colonies are formed on the bottom of the culture flask. In sugar nutrient solutions it produces a sediment of yeast cells and a thin film on the surface of the liquid. No spores are formed.

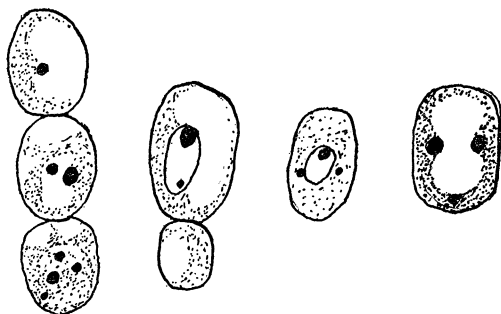


Fig. 3. Culture 3. Cells $\times 2000$.

Culture 4, Torula sp?—This white species of *Torula*, obtained from elderberry wine spontaneously fermented, develops in the same manner as the cultivated yeasts. The cells (fig. 4) are oval, $3-4 \times 4-5\mu$ in diameter. Cultures in yeast water containing saccharose, dextrose, levulose, maltose,

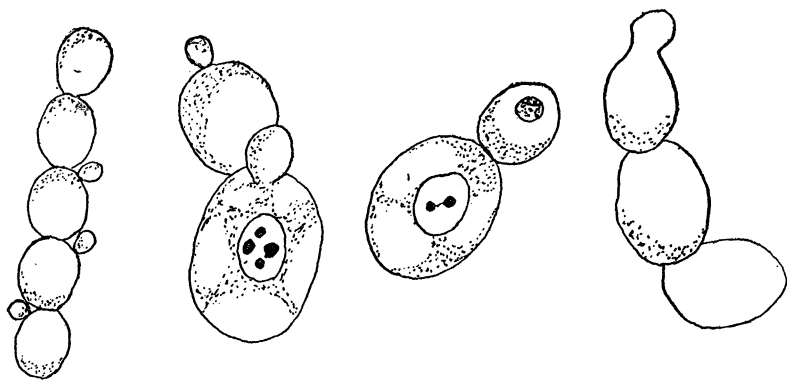


Fig. 4. Culture 4. Cells $\times 2000$.

and lactose, develop rapidly, with a sediment of yeast cells and the formation of a thin film on the surface of the culture liquid. In yeast water containing succinic, citric, or acetic acids, the film on the surface of the culture liquid is white, opaque, and wrinkled. In the presence of tartaric and

malic acids the separate colonies are connected into a net-like film. Gelatin is not liquefied, and no spores are formed.

Culture 5, Torula sp?—The cells of this species (fig. 5), obtained from the nectar of *Oenothera sp?*, are oval to elliptical,

2–2.5 $\times$ 4–5 μ .

On beer-wort agar and gelatin the colonies are small, white, and spherical. In sugar nutrient solutions and in yeast water containing citric, malic, or lactic acids, there is

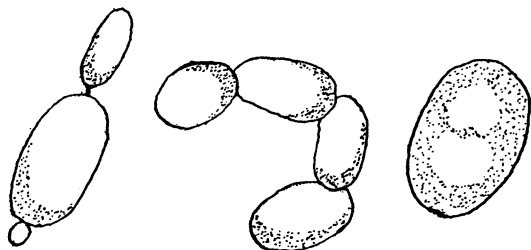


Fig. 5. Culture 5. Cells $\times$ 2000.

a considerable sediment of yeast cells and a thin film on the surface of the culture liquid. In yeast water containing tartaric acid the colonies remain more or less distinctly formed on the bottom of the culture flask. No spores are produced, and gelatin is not liquefied.

Culture 6, Torula sp?—The colonies of this species of *Torula*, obtained from the seeds of *Zea Mays*, may be distinguished from cultures

4 and 5 by the growth appearance on agar and gelatin. The margin of the colonies is irregular and extends radially from the center of growth. The cells (fig. 6) are smaller, being 1.5–2.5 $\times$ 2–3.5 μ . Multiplication is by budding, more particularly at the ends of the mother cells. In nutrient solutions

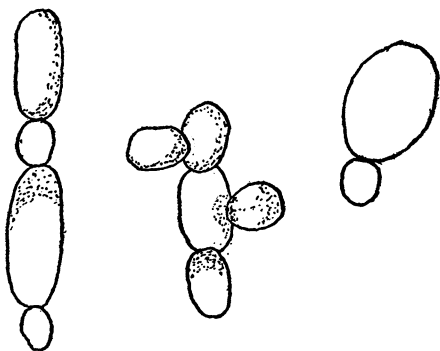


Fig. 6. Culture 6. Cells $\times$ 2000.

containing galactose and levulose, development takes place rapidly, with the formation of a sediment of yeast cells and a very dense wrinkled film on the surface of the liquid. In nutrient solutions containing maltose and lactose, the film is

made up of a network of distinct colonies, but in the presence of saccharose the film is slimy and stringy in appearance. Dextrose and levulose are fermented. Gelatin is not liquefied, and spores are not formed.

Culture 7, Alternaria sp?—Colonies of this organism, obtained from the fruit of *Ribes Grossularia*, on gelatin or agar plates, appear much like the colonies of wild yeasts. At first they are made up of budding cells; later a few radiating strands of mycelium appear, with budding conidia at or near the cross walls. The culture soon becomes black from the formation of chlamydospores. The growth on

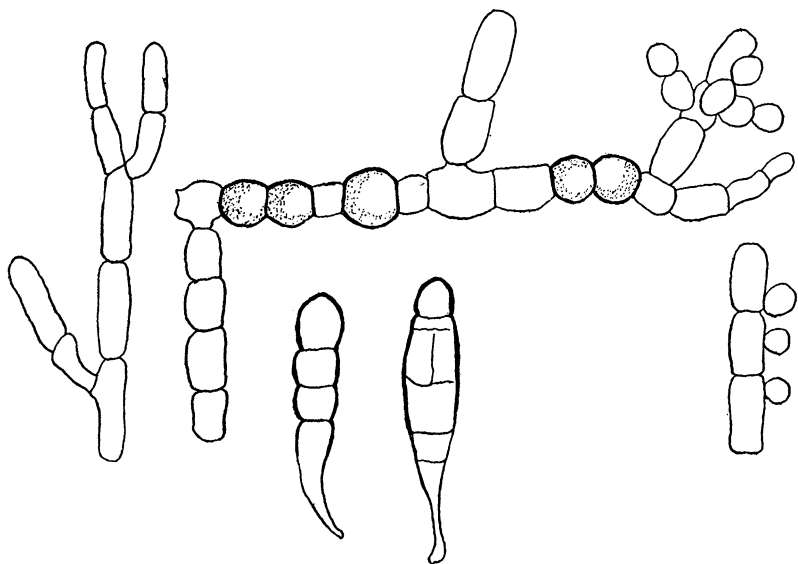


Fig. 7. Culture 7. Vegetative cells, conidia, chlamydospores, and muriform spores.

agar after seven days, results in the appearance of four distinct regions to each colony. The central part is black and shiny with a slightly wrinkled surface. About this area are three zones concentrically arranged and of a very dark green color, the terminal margin being made up of a filamentous growth.

In nutrient sugar solutions, the film is at first white then black. Dextrose and saccharose are fermented. In yeast

water containing organic acids, there is a sediment of yeast cells and a film of anastomosing colonies. Gelatin is liquefied. The cells (fig. 7) vary in size from $6-8 \times 8-10\mu$. Besides the single-celled chlamydospores, there are occasionally many-celled spores borne on short conidiophores. The septa in the spore occur both at right angles and parallel to the long axis and are muriform.

Culture 8, Alternaria sp?—The colonies of this culture are at first made up of a central mass of yeast cells with my-

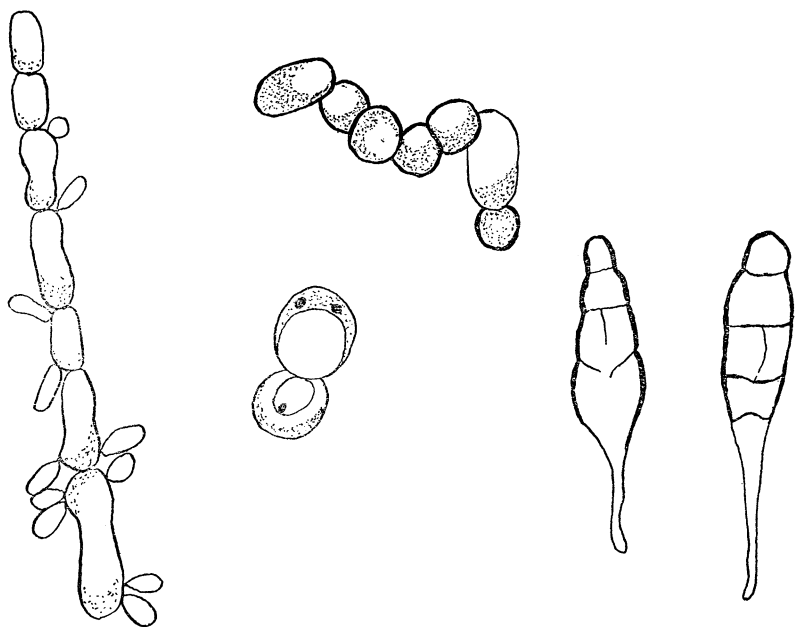


Fig. 8. Culture 8. Vegetative cells, conidia, chlamydospores, and muriform spores.

celium extending radially from the center. Budding conidia are rapidly formed at regular intervals on this mycelium. The cells (fig. 8) vary in size from $3-6 \times 4-9\mu$. After seven days' growth on agar, the colonies may be separated into a central flat disc and several distinct zones, these zones gradually changing from a dark green to a black color. In sugar nutrient solutions there is a rapid growth and a very thick, slimy, greenish black film. Dextrose, saccharose, and

maltose are fermented. In yeast water containing organic acids, growth is as rapid as in the sugar nutrient solutions; but instead of forming a film, the growth on the surface is limited to the margin along the side of the culture flask. The cells vary in size from $2 \times 10\text{--}25\mu$ or $3 \times 7\text{--}8\mu$. Gelatin is liquefied. Chlamydospores and muriform many-celled spores occur in acid yeast-water solution.

Culture 9, Oospora sp?—The colonies of this organism appear much like certain white yeasts. They are circular in outline and have a more or less wrinkled surface. Growth of this organism does not take place by budding; but the mycelium, which is dichotomously branched, predominates

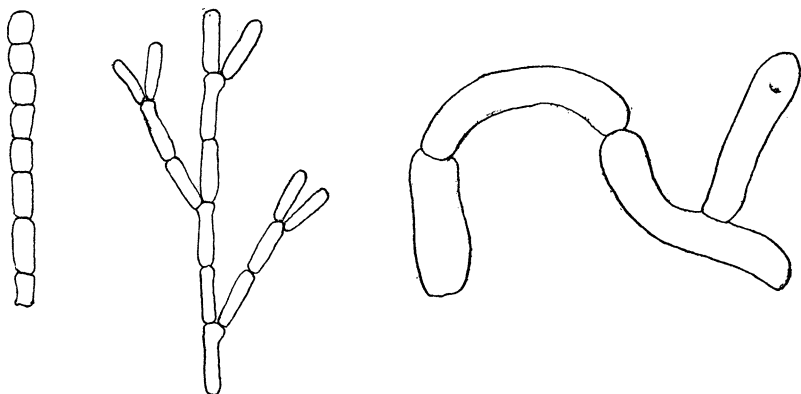


Fig. 9. Culture 9. Vegetative cells.

in all media. The cells (fig. 9) are elongated, $2\text{--}2.5 \times 12\text{--}15\mu$, or short and rectangular, $3\text{--}4 \times 4\text{--}6\mu$. In yeast water containing organic acids, certain of the cells may become quite large and sickle-shaped, $4\text{--}5 \times 20\text{--}30\mu$. No chlamydospores or conidia are found in any of the cultures. When grown in Raulin's nutrient sugar solutions the liquid becomes bright red in color. This characteristic easily distinguishes this organism from the other cultures with mycelial growth. Gelatin is liquefied.

Culture 10, Alternaria sp?—This organism growing on agar forms a membrane of yeast cells and mycelium of a flesh-pink color. The yeast cells (fig. 10) vary in size from

$4-8 \times 5-8\mu$. The culture does not become dark in any media and rarely forms chlamydospores. In yeast water containing organic acids only a few chlamydospores are found among the cells on the surface of the liquid. In nutrient sugar solutions and in yeast water containing organic acids the

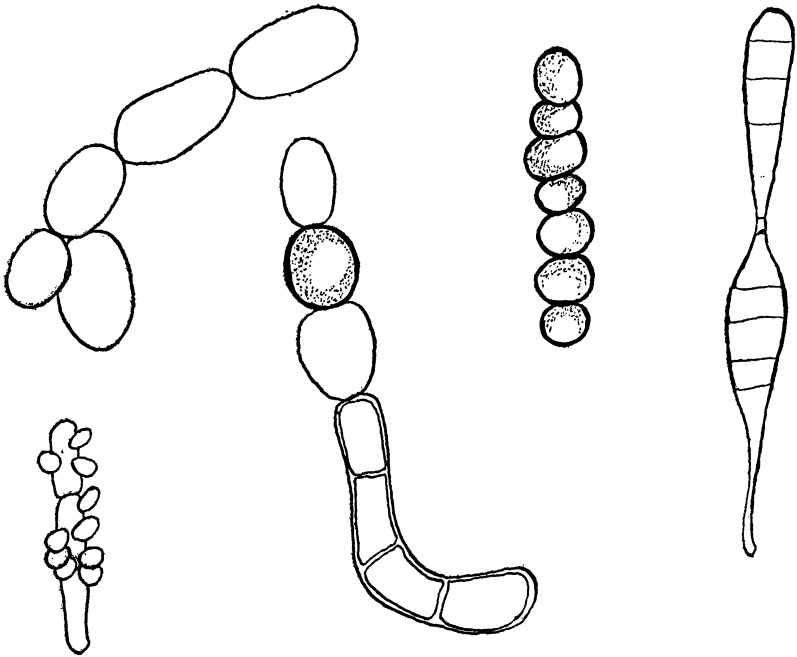


Fig. 10. Culture 10. Vegetative cells, conidia, chlamydospores, and muriform spores.

vegetative growth results in the formation of a white, glistening mass of fungous elements that cannot be separated easily. Only a few muriform many-celled spores are found, which indicates its being a species of *Alternaria* that does not readily form spores in culture.

Culture 11, Alternaria sp?—This organism liquefies gelatin more rapidly and becomes black—due to the formation of chlamydospores—more quickly than any of the fungi previously described. On gelatin or agar it first appears in irregular yeast-like colonies. The budding conidia develop rapidly; and if the colonies on the surface of the media

are crowded, only yeast cells are formed. Saccharose and maltose are fermented. In yeast water containing citric, tartaric, or succinic acids, the sediment is made up of yeast cells, whereas the black, leathery film is composed of mycelia and chlamydospores. The cells (fig. 11) vary in size from $4-6 \times 10-12\mu$ or $2 \times 10-50\mu$. The many-celled muriform spores are found in cultures grown in acid yeast-water solutions.

Culture 12, Alternaria sp?—This fungus is differentiated in part from the other black yeast-like fungi by the late ap-

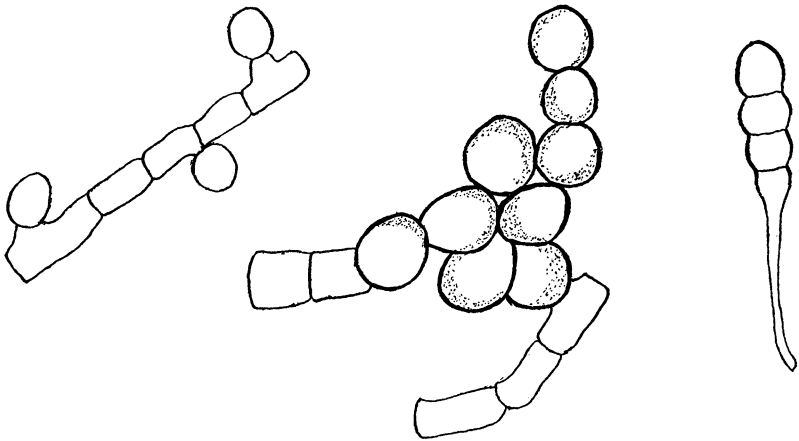


Fig. 11. Culture 11. Vegetative cells, chlamydospores, and muriform spores.

pearance of chlamydospores and the predominance of yeast cells. In early cultures on agar the colonies are yeast-like in appearance, and the mycelium starts to develop only after the medium has become less moist. In nutrient solutions containing sugars the growth is very rapid, and a ring of colonies appears on the surface. In yeast water containing organic acids there is a sediment of yeast cells, or the growth is limited to isolated colonies on the bottom of the flask. The ring appearing on the surface of the liquid is white in color except in the presence of lactic acid, in which case it is black. This fungus, a species of *Alternaria*, has dark chlamydospores and many-celled muriform spores. The cells (fig. 12) vary in size from $3-4 \times 5-50\mu$. Gelatin is

rapidly liquefied. Saccharose, dextrose, levulose, and maltose are fermented.

Culture 13, Saccharomyces cerevisiae.—This yeast, obtained from "Yeast Foam," has spherical cells (fig. 13) varying in size from 5 to 9 μ in diameter. Growth takes place by budding from all sides of the mother cell. In nutrient sugar solutions the organism forms a sediment of yeast cells and a thin film only in the presence of glucose. In yeast water containing organic acids, the sediment of yeast cells has a flocculent appearance. Saccharose, dextrose, levulose, and

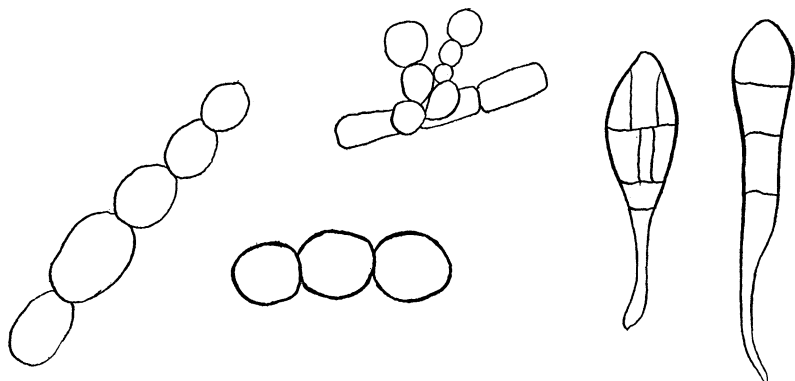


Fig. 12. Culture 12. Vegetative cells, conidia, chlamydospores, and muriform many-celled spores.

maltose are fermented, with the formation of alcohol and carbon dioxide. Spores are formed on moist porous plates, on Gorodkova's test medium, and on yeast-water agar. On the latter medium, after a growth of ten days, 30–40 per cent of the yeast cells have formed endospores. The number of ascospores in the asci varies from 1 to 4, and they vary in size from 2.5 to 4 μ in diameter. Upon germination the spores enlarge and are set free from the spore case and then develop separately, or the spores may fuse as they become larger, the spore case becoming thinner at the same time, which results in a very large cell that develops by budding. Sometimes the ascospores start to germinate before they are set free from the ascus.

The reaction of the thirteen organisms was tested in nutrient solutions of saccharose, dextrose, levulose, and mal-

tose. The sugar nutrient solutions contained 1 per cent peptone, .3 per cent monopotassium phosphate, and .02 per cent magnesium sulphate. The concentration of the sugar was 5 per cent saccharose, 5 per cent dextrose, 1 per cent levulose, and 1 per cent maltose, respectively.

For the determination of alcohol formation, 100 cc. of the sugar nutrient solutions were placed in 125-cc. Erlenmeyer

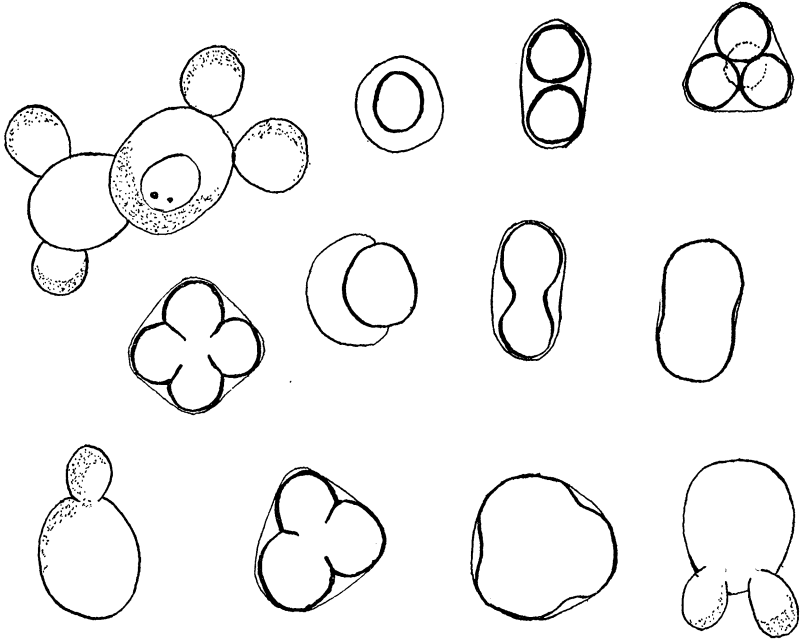


Fig. 13. Culture 13. Vegetative cells, asci with ascospores, and spores during germination.

flasks, and after sterilization each was inoculated with a pure culture of one of the thirteen different strains and incubated at room temperature for thirty days. Immediately after the inoculation of the nutrient solutions the cotton plugs in the culture flasks were replaced by one-holed rubber stoppers, through which passed a piece of small glass tubing with a small cotton plug. This precaution was taken to prevent excessive evaporation. After the incubation at room temperature for thirty days, the nutrient solutions were neutralized with normal caustic soda and made up to the

original volume with distilled water. The loss by evaporation did not exceed .3 cc. in any case. Fifty cc. were then distilled off, and the amount of alcohol present determined with a pycnometer. The presence of alcohol was confirmed in each case by means of the iodoform test as used by Will ('10) in his investigation on certain species of *Mycoderma*.

TABLE I

SHOWING THE PERCENTAGE, BY VOLUME, OF ALCOHOL PRODUCED BY THE THIRTEEN DIFFERENT CULTURES AFTER INCUBATION AT ROOM TEMPERATURE FOR THIRTY DAYS

Culture no.	Saccharose	Iodoform test	Dextrose	Iodoform test	Levulose	Iodoform test	Maltose	Iodoform test
1	.10	—*	.10	—	.10	—	.30	—
2	.05	—	.05	—	.10	—	.30	—
3	.00	—	.10	—	.10	—	.00	—
4	.15	—	.00	—	.10	—	.20	—
5	.00	—	.00	—	.10	—	.05	—
6	.00	—	.85	+	.30	+	.10	—
7	.95	+	.65	+	.15	—	.25	—
8	1.20	+	.45	+	.10	—	.45	+
9	.00	—	.00	—	.05	—	.20	—
10	.10	—	.10	—	.05	—	.00	—
11	.95	+	.35	—	.15	—	.55	+
12	.80	+	1.00	+	.50	+	.75	+
13	5.35	+	5.00	+	.70	+	.85	+
Control (without organism)	.00	—	.00	—	.00	—	.00	—

*The sign + indicates that the iodoform test for alcohol was positive, with the presence of iodoform crystals.

The results, as indicated by table I, show that very little alcohol, if any, is formed by the various cultures with the exception of culture 13 (*Saccharomyces cerevisiae*), which was used as a control. Only one culture of *Torula*, culture 6, produced alcohol by the fermentation of dextrose and levulose. Four of the black yeast-like fungi-cultures, 7, 8, 11, and 12, produced only small quantities of alcohol.

The reaction of the thirteen different cultures was tested in yeast water containing organic acids. Seventy-five grams of press-yeast were ground up with enough distilled water to form a thin paste. More distilled water was then added

to make up the volume to one liter. This yeast suspension was then heated in an Arnold sterilizer at 100°C. for one hour. After most of the yeast cells had settled to the bottom of the flask, the supernatant liquid was decanted and filtered through hard filter paper. This filtrate was then run through a Berkfeld cylinder to remove all yeast cells. The acid nutrient solutions were made from this last filtrate by the addition of 1 per cent citric, 1 per cent lactic, 1 per cent malic, 1 per cent succinic, 1 per cent tartaric, and .5 per cent acetic acid.

For the acid reaction 50 cc. of the acid nutrient solutions in 100-cc. Erlenmeyer flasks were sterilized, and then each was inoculated with a pure culture from one of the thirteen fungi. After incubation for thirty days at room temperature, the solutions were made up to the original volume with distilled water. A 10-cc. portion was then titrated with N/10 caustic soda, phenylphthalein and litmus being separately used as indicators. The results given in table II are in terms of cubic centimeters of N/10 caustic soda required to neutralize 10 cc. of the culture solutions.

TABLE II

SHOWING THE FINAL ACID REACTION AND THE CHANGE IN REACTION THAT HAD TAKEN PLACE AFTER THE CULTURES WERE INCUBATED AT ROOM TEMPERATURE FOR THIRTY DAYS

Culture no.	Acid	Phenylphthal- ein as indicator	Difference	Litmus as indicator	Difference
Control	Acetic	6.3*	—	5.6	—
	Citric	6.55	—	5.7	—
	Lactic	4.5	—	3.8	—
	Malic	6.15	—	5.45	—
	Succinic	6.5	—	5.9	—
	Tartaric	6.0	—	5.5	—
1	Acetic	6.5	+ .2	5.7	+ .1
	Citric	5.5	—6.0	—3	—6.0
	Lactic	3.0	—1.5	2.3	—1.5
	Malic	3.5	—2.65	2.8	—2.65
	Succinic	2.9	—3.6	2.3	—3.6
	Tartaric	5.1	— .9	4.5	—1.0
2	Acetic	6.7	+ .4	6.1	+ .5
	Citric	.3	—6.25	—3	—6.0
	Lactic	.25	—4.25	—5	—4.3
	Malic	.3	—5.85	—3	—5.75
	Succinic	.3	—6.2	—5	—6.4
	Tartaric	3.8	—2.2	3.3	—2.2
3	Acetic	6.3	.0	5.7	+ .1
	Citric	4.1	—2.45	3.3	—2.4
	Lactic	3.4	—1.1	2.7	—1.1
	Malic	.7	—5.45	.2	—5.25
	Succinic	.5	—6.0	—4	—6.3
	Tartaric	5.2	— .8	4.5	—1.0
4	Acetic	.1	—6.2	—5	—6.1
	Citric	.45	—6.1	—3	—6.0
	Lactic	.4	—4.1	—3	—4.1
	Malic	.35	—5.8	—3	—5.75
	Succinic	.3	—6.2	—3	—6.2
	Tartaric	6.2	+ .2	5.5	.0
5	Acetic	6.65	+ .35	5.9	+ .3
	Citric	.9	—5.65	.2	—5.5
	Malic	2.65	—1.85	2.0	—1.8
	Lactic	.3	—5.85	—3	—5.75
	Succinic	.4	—6.1	—3	—6.2
	Tartaric	5.7	— .3	5.0	— .5

*The results are given in terms of cc. N/10 caustic soda required to neutralize 10 cc. of the culture solutions; and the change in reaction, whether an increase or decrease, is indicated in terms of cc. N/10 caustic soda per 10 cc. of the culture solution.

TABLE II. (Continued)

Culture no.	Acid	Phenylphthal- ein as indicator	Difference	Litmus as indicator	Difference
6	Acetic	6.65	+ .35	5.8	+ .2
	Citric	.3	-6.25	— .4	-6.1
	Lactic	.7	-3.85	.2	-3.6
	Malic	.2	-5.95	— .4	-5.85
	Succinic	.4	-6.1	— .2	-6.1
	Tartaric	5.4	— .6	4.9	— .6
7	Acetic	6.9	+ .6	6.1	+ .5
	Citric	.4	-6.15	— .3	-6.0
	Lactic	1.6	-2.9	1.0	-2.8
	Malic	.1	-6.05	— .5	-5.95
	Succinic	.0	-6.5	— .6	-6.5
	Tartaric	.5	-5.5	— .3	-5.8
8	Acetic	7.7	+1.4	6.8	+1.2
	Citric	.3	-6.25	— .4	-6.1
	Lactic	1.6	-2.9	1.2	-2.6
	Malic	.2	-5.95	— .5	-5.95
	Succinic	.0	-6.5	— .6	-6.5
	Tartaric	.3	-5.7	— .3	-5.8
9	Acetic	6.9	+ .6	6.0	+ .4
	Citric	6.8	+ .25	6.1	+ .4
	Lactic	4.6	+ .1	4.1	+ .3
	Malic	6.0	+ .15	5.3	+ .15
	Succinic	6.3	— .2	5.4	— .5
	Tartaric	7.5	+1.5	7.0	+ .5
10	Acetic	7.6	+1.3	7.0	+1.4
	Citric	5.3	-1.25	4.5	-1.2
	Lactic	3.1	-1.4	2.4	-1.4
	Malic	3.3	-2.85	2.5	-2.95
	Succinic	.4	-6.1	— .3	-6.2
	Tartaric	6.0	0.0	5.6	+ .1
11	Acetic	6.9	+ .6	6.2	+ .6
	Citric	.2	-6.35	— .6	-6.3
	Lactic	1.3	-3.2	.7	-3.1
	Malic	.2	-5.95	— .4	-5.85
	Succinic	.0	-6.5	— .6	-6.5
	Tartaric	.4	-5.6	— .2	-5.7
12	Acetic	7.6	+1.3	6.8	+1.2
	Citric	3.8	-2.75	3.0	-2.7
	Lactic	3.1	-1.4	2.5	-1.3
	Malic	.5	-5.65	— .1	-5.55
	Succinic	.2	-6.3	— .4	-6.3
	Tartaric	5.3	— .7	4.8	— .7
13	Acetic	6.6	+ .3	5.8	+ .2
	Citric	5.5	+1.05	4.7	+1.0
	Lactic	2.3	-2.2	1.8	-2.0
	Malic	4.4	-1.75	3.7	-1.75
	Succinic	4.2	-2.3	3.8	-2.1
	Tartaric	6.4	+ .4	5.8	+ .3

TABLE III

SHOWING THE CHANGE IN ACID REACTION BROUGHT ABOUT BY THE THIRTEEN CULTURES DURING THE INCUBATION AT ROOM TEMPERATURE FOR THIRTY DAYS

(Results are the averages of the two titrations given in table II)

Culture no.	Acetic	Citric	Lactic	Malic	Succinic	Tartaric
1	+ .15*	-6.0	-1.5	-2.65	-3.6	-.95
2	+ .45	-6.1	-4.3	-5.8	-6.3	-2.2
3	.0	-2.4	-1.1	-5.35	-6.15	-.9
4	-6.15	-6.05	-4.1	-5.8	-6.2	+ .1
5	+ .3	-5.6	-1.8	-5.8	-6.15	-.4
6	+ .25	-6.2	-3.7	-5.9	-6.1	-.6
7	+ .55	-6.1	-2.85	-6.0	-6.5	-5.65
8	+1.3	-6.15	-2.75	-5.95	-6.5	-5.65
9	+ .5	+ .3	+ .2	+ .15	-.35	+1.5
10	+1.35	-1.2	-1.4	-2.9	-6.15	0.0
11	+ .6	-6.3	-3.15	-5.9	-6.5	-5.65
12	+1.25	-2.7	-1.35	-5.6	-6.3	-.7
13	+ .25	+1.0	-2.1	-1.75	-2.2	+3.5

*In terms of N/10 caustic soda per 10 cc. culture solution.

The results, as indicated by tables II and III, show that all the cultures produce a change in the reaction of the acid yeast-water solutions after incubation at room temperature for thirty days. There is a decrease in the acidity of all the acid nutrient solutions except in the presence of acetic acid, in which case a marked decrease in acidity was brought about only by culture 4. In the case of culture 9, only a slight change of acidity was found in any of the acid nutrient solutions.

ANIMAL EXPERIMENTS

The experiments on animals were made in the laboratory of the pathological department of the Washington University Medical School, under the direction of Dr. E. L. Opie and Dr. W. S. Thomas. Rabbits and guinea-pigs were inoculated with suspensions of the different yeast-like fungi, with the exception of cultures 3 and 13, either from cultures grown in Hansen's solution for 12 hours, or from 48-hour cultures grown on agar and suspended in Ringer's physiological salt solution.¹ In each case 2 cc. were injected into the

¹Ringer's solution contains the following:

Sodium chloride.....	.7 per cent	Calcium chloride.....	.028 per cent
Potassium chloride....	.035 per cent	Sodium carbonate.....	.003 per cent

marginal ear vein of rabbits, or 1 cc. intraperitoneally or subcutaneously in the guinea-pigs. All injections were made under aseptic conditions with sterilized instruments.

The animals that died were carefully examined for lesions or abnormalities, the autopsy being carried out with aseptic precautions with sterilized instruments. At the same time a sample of blood was taken from the right auricle of the heart, with a sterilized platinum loop, and transferred to a tube containing sterilized beer-wort. All the principal organs were removed to sterilized Petri dishes, and then small pieces of the liver, lungs, spleen, kidneys, and intestines were placed in tubes of sterilized beer-wort. These tubes were then incubated at 28°C. for seventy-two hours, but were kept under observation for over ten days, and then gelatin plates made from them.

Where the organisms had been inoculated into beer-wort and the same procedure used as for the animal tissues, pure cultures were again easily obtained on gelatin plates with all the eleven strains used.

At the same time pieces of the different organs were fixed in Bouin's or Gilson's fluid, imbedded in paraffin, sectioned, and stained with saffranin and methyl blue, or with Delafield's hematoxylin and eosin, or according to the method used by Pianezze ('96) for the staining of carcinoma tissue.

TABLE IV
RESULTS OF ANIMAL EXPERIMENTS

Experiment number	Animal number	Kind of animal	Sex	Culture number	Kind of culture	Kind of injection	Days until death	Cause of death	Lesions	Source of organism	Stained preparations
1	1	G-p*	M*	1	R*	P*		Killed		None	Negative
2	2	r*	M	2	H*	V*	56	Unknown	White spots on liver	Intestines	Lung air spaces condensed
	3	G-p	M	2	H	S*	35	Unknown	None	None	Negative
	4	G-p	M	2	H	P	49	Unknown	None	None	Negative
	5	G-p	M	2	R	P		Killed	None	None	Negative
3	6	G-p	F*	4	H	S		Killed	None	None	Negative
	7	G-p	M	4	H	P	7	Unknown	None	Intestines	Lung air spaces condensed
	8	G-p	M	4	H	P	17	Unknown	None	None	Negative
	9	G-p	F	4	R	P		Killed	None	None	Negative
	10	G-p	F	4	R	P		Killed	None	None	Negative
4	11	r	M	5	H	V	1½	Unknown	None	None	Negative
	12	r	M	5	R	V	½	Killed	None	None	Negative
	13	r	M	5	R	V		Killed	None	None	Negative
	14	G-p	M	5	H	S		Killed	None	None	Negative
	15	G-p	F	5	H	P	60	Unknown	None	None	Negative
5	16	G-p	M	6	H	S		Unknown	None	None	Negative
	17	G-p	M	6	H	P	16	Unknown	None	None	Negative
	18	r	F	6	H	V		Killed	None	None	Negative
6	19	G-p	M	7	R	P	20	Unknown	None	None	Negative
	20	r	M	7	b-w*	V					
7	21	G-p	M	8	R	P	20	Unknown	None	None	Negative
	22	r	M	8	b-w	V					
8	23	r	M	9	H	V	60	Unknown	White spots on liver	None	Negative
	24	G-p	F	9	H	P		Unknown	None	None	Negative
	25	G-p	M	9	R	P		Unknown	None	None	Negative
9	26	G-p	M	10	R	P	10	Unknown	None	None	Negative
	28	r	M	10	b-w	V					
10	27	G-p	M	11	R	P	20	Unknown	None	None	Negative
	29	r	F	12	H	V		Killed	None	None	Negative
	30	G-p	M	12	H	S		Killed	None	None	Negative
	31	G-p	M	12	H	P	20	Unknown	None	Intestines and lungs	Lung air spaces condensed
11	32	G-p	M	12	R	P		Killed	None	None	Negative
	33	G-p	M	12	b-w	P	3	Unknown	None	None	Negative
	34	r	M	12	b-w	V					

*G-p represents guinea-pig; r, rabbit; M, male; F, female; H, suspension in Hansen's solution; R, suspension in Ringer's solution; P, intraperitoneal; S, subcutaneous; V, intravenous; b-w, sterilized in beer-wort.

TABLE V
SHOWING THE WEIGHT IN GRAMS OF THE ANIMALS WEEK BY WEEK*

Animal no.	Initial wt.	Weight at end of the following periods:								Remarks
		1st wk.	2nd wk.	3d wk.	4th wk.	5th wk.	6th wk.	7th wk.	8th wk.	
1	*555	500	475	531	520	550	535			570 gms. after 5 months.
2	2450	2400	2370	2300	2280	2170	2100	2010	1810	Died 9 days after 8th wk.
3		375	333	320	310	275				Died after 4 wks. 5 days.
4		435	420	410	449	402	402	340		Died after 6 wks. 5 days.
5	570	490	470	520	495					515 gms. after 4 months.
6	385	365	370	370	405	400	400	410	380	450 gms. after 4 months.
7	390	290								Died after 7 days.
8	535	475		435						Died after 2 wks. 3 days.
9	560	480	515		485	560		615		620 gms. after 4 months.
10	570	495	500		475	475		515		520 gms. after 4 months.
11	2050	2030								Died after 9 days.
12	2600	2400		2300	2240			2110		Killed after 2 months.
13	1700	1550		1635	1490			1745		Killed after 2 months.
14	395	370	380	375	405	380	385	395	350	400 gms. after 3 months.
15	375	350	372	372	400	380	355	380	317	Died after 2 months.
16	375	355	345	335	365	355	355	365	375	400 gms. after 4 months.
17	265	240	240	235	240	225	220			Died after 5 wks. 5 days.
18	1670	1575	1690	1620	1750	1700	1540	1680	1820	1690 gms. after 4 months.
19	555	510		405						Died after 2 wks. 5 days.
20	660									Living.
21	530	475		395						Died after 2 wks. 5 days.
22	780									Living.
23	1350	1400	1340	1390	1430	1430	1430	1460	1420	1365 gms. after 9 wks.
24		382	380	325	375	375	390	410	415	Killed after 2 months.
25	500	440		420	460		435		420	Killed after 2 months.
26	465		395							Died after 10 days.
27	470	445		400						Died after 20 days.
28	870									Living.
29	1300	1280	1240	1295	1250	1250	1385	1350	1415	Killed after 2 months.
30		345	325	310	338	342	359	325	345	Killed after 2 months.
31		311	300	280						Died after 20 days.
32	470		385	420	415		455	450		535 gms. after 4 months.
33	410	360								Died after 3 days.
34	920									Living.

*Each animal was weighed once a week from the time of inoculation to the time of death.

The results of experiment 1 were negative.

In experiment 2 animal No. 2, white spots were found on the liver. These lesions were not caused by the organism injected, for inoculations were taken directly from these areas but no fungous culture was obtained. Rabbits are frequently infected by coccidia, and it is probable that they were the cause of the lesions in this animal. Stained

preparations revealed a condensation of the air spaces of the lungs. There were no organisms present and this condition was probably due to post-mortem changes such as oedema.

In experiment 3 animal No. 7 died after seven days, and the autopsy revealed the intestines very much inflamed. The organism was isolated from the intestines, and stained preparations showed abnormalities in the condensation of air spaces in the lungs. A repetition of this experiment gave negative results.

In experiment 4 animal No. 11 died in less than two days from causes that could not be determined. The death of this animal may have been due to the fact that phagocytosis was not sufficiently active, since a repetition of the same experiment gave negative results.

In experiment 5 the results were negative.

In experiment 6 animal No. 19 died after twenty days without evidence of lesions. To test out the production of toxin by culture No. 7, 1 cc. of a four-day culture in beerwort after sterilization was injected intravenously into a 660-gram rabbit, but the results were negative.

In experiment 7 animal No. 21 died after twenty days from unknown causes. The condensation of air spaces found in the lungs was probably due to post-mortem changes. Animal No. 22, a 780-gram rabbit, was subjected to a 1-cc. sterilized injection of a four-day culture of organism No. 8, with negative results.

In experiment 8 animal No. 23 died after two months without evidence of lesions other than white spots on the liver which were presumably due to coccidia, since no fungous culture was obtained from this lesion.

In experiment 9 animal No. 26 died after ten days from unknown causes. Intravenous injection in an 870-gram rabbit of a 1-cc. four-day sterilized culture grown in beerwort, gave negative results.

In experiment 10 animal No. 27 died after twenty days without evidence of lesions or the presence of fungi in the various organs.

In experiment 10 animal No. 31 died after twenty days. The organism injected was again isolated from the intestines and the lungs. The condensation of air spaces in the lungs of this animal was probably due to post-mortem changes. A repetition of this experiment gave negative results, as shown by animal No. 32 which lived four months without evidence of any injurious effects due to an organism. Animal No. 33 received a 1-cc. injection of a two-day sterilized culture of organism No. 12, grown in beer-wort. This animal died in three days. However, a 2-cc. intravenous injection of a two-day sterilized culture of organism No. 12, grown in beer-wort, gave negative results.

All the remaining twenty-four animals inoculated gave negative results, in that no evidence was found of an injurious effect due to the cultures injected.

DISCUSSION

There has been a diversity of opinion in regard to the importance of fungi as agents in the production of infectious diseases. More recent investigations indicate that fungi are of secondary importance in the formation of lesions in animal bodies, and usually appear secondarily in infected tissues. On the other hand, certain species of fungi are to be considered of primary importance in those cases in which they prove toxic to animals if consumed in large quantities on infected foods.

It is easy to recognize that the parasitic fungus cannot prosper with the same degree of success on all animal species. *Mucor* and *Aspergillus* pneumonomycoses, observed frequently in birds, is rarely found in other animals. Certain varieties of *Sporotrichum* (*Microsporon*) which occur on infants, seem to grow with difficulty on animals. Certain varieties of *Oospora* (*Trichophyton*) are common to man and other animals, whereas still other varieties of *Oospora* appear only on man. The changes brought about by the disease-producing organisms in the body are quite varied, differing quite as much as the morphological and cultural characters of the organism when grown outside the body.

In close connection with the anatomical changes produced in the body a study should be made of the physiological relations of host and parasite, more particularly the conditions which predispose the body to attack. Sticker ('00) observed that mycosis in man may be of sporadic or of endemic origin. In the former case weakened individuals suffering from other diseases are attacked. Out of thirty-nine cases of mycosis only five occurred in persons supposedly in good health. The endemic disease appears in consequence of the patient's vocation: for example, the pigeon caretaker, hair-dressers in Paris, and the sponge purifiers.

The diseases produced by fungi, in proportion to the wide distribution of parasitic species, are of rare occurrence in man. Siebenmann, who investigated the distribution of *Aspergillus fumigatus* from the literature on otomycosis, discovered that, while it appeared in all parts of Europe and America, it was most abundant in India, its frequency depending on the time of the year.

The experimental results with injections of certain species of the *Phycomycetes* have been mostly negative. All authentic instances of subcutaneous injections have given negative results, whereas only a few species of the *Phycomycetes* produce death in animals by intraperitoneal or intravenous injections. The resulting lesions in the rabbits and guinea-pigs vary with the manner of injection, the kidneys and mesenteric glands being regularly altered. An injection of a 2-cc. spore-suspension of a non-toxic fungus (*Sterigmatozystis nigra*) will result in the animal's losing 10 to 25 per cent in weight during the first three or four days after the injection; after this period the animal rapidly regains its original weight. If the amount of injection is increased, the animal will die from mechanical causes in about eight days. Sections of the kidneys show that the spores have collected in the glomeruli, where some of the spores germinate but no multiplication of cells takes place. The heart and blood remain sterile.

Ballin ('08) subjected animals to five-day cultures of certain moulds. He then killed the animals after a few hours

and investigated the lungs microscopically. According to this author, if guinea-pigs are allowed to breathe the spores of *Aspergillus fumigatus* grown on agar plates, they die within seven or eight hours by asphyxia. Upon examination of the lungs of the dead animals, he had no difficulty in finding the spores in hemorrhagic colonies which permeated the lungs. The same result was obtained with cultures of *Sterigmatocystis nigra*. The germination of the spores takes place in the alveolar septa between the alveoli, and then by means of pressure the germinating spores break through the cell walls and enter the alveoli.

Fungous infectious agents are not absolutely deprived of the power to secrete toxic substances, although this toxicity does not seem to be as evident in them as in the bacteria. Lucet was among the first to mention the existence of a thermolabile toxic substance in cultures of *Aspergillus fumigatus*. The earlier investigators assert that the intensity of the toxic action of certain fungi is proportional to the quantity of the fungous spores injected, and in this manner the higher fungi differ from pathogenic bacteria, in which the resultant intensity of toxic action is to a large extent independent of the number of bacteria injected into the animal.

Ceni ('07) found that the toxic action of an alcoholic or ether extraction from cultures of *Aspergillus fumigatus* was of a specific character. He isolated a culture of *Aspergillus* from the atmosphere in the home of a family affected with chronic pellagra, and obtained a water-soluble toxin from the cultures of this fungus. He also isolated two toxic varieties of *Penicillium* from corn, the toxic substance from one variety producing neuromuscular condensations in animals, whereas the toxic substance in the other produced nervous depression. Bodin and Gautier ('06) also found that this toxin was not destroyed by heating to 120°C. for thirty minutes. Otto ('07) obtained an alcoholic extract from five strains of *Aspergillus fumigatus* which was toxic for animals either by intraperitoneal injection, or as an emulsion if washed into the stomach by means of a probe. Not only

the spores but also the mycelium contained substances of intensive poison. Sturli ('10) extracted a toxin from cultures of *Penicillium glaucum*, which was neither a phenol, an acid, or an alkaloid.

Blakeslee and Gartner ('13) found that the "presssaft" from the aërial filaments of *Rhizopus nigricans* caused almost instant death when injected intravenously into rabbits. Several other species of *Mucorineae* were tested but with negative results. A solution containing the water-soluble substance extracted from .045 grams of the dry fungus, when injected intravenously, is sufficient to kill a 1.35-kilogram rabbit in less than two minutes. The poison from *Rhizopus* appears to be 5.5 times that of the tubercle bacillus, 15 times that obtained from edestin, and 45 times that of penicillic acid. *Rhizopus nigricans* is widely distributed, and is almost certain to appear as a spontaneous infection on bread and similar substrata rich in carbohydrates whenever the proper temperature and moisture requirements are observed. Blakeslee and Gartner point the possible relation of this fungus to diseases of unknown origin, such as pellagra, horse disease, and the cornstalk disease of the Middle West, all of which have been attributed to infected food.

Mohler ('14), in a review of the investigations on cerebrospinal meningitis (forage-poisoning), emphasized the widely accepted theory that this disease may be due to fungi on the feed. While most investigators have obtained negative results, Mays reported that a colt fed experimentally upon some of the mouldy corn which was held responsible for the serious outbreak in Kansas in 1890, developed the disease on the twenty-sixth day. Again, the Kansas outbreak in 1906 was said by Haslam to have been caused by the consumption of immature ears of corn infected by moulds, although the exact mould was not determined. By feeding horses upon this corn, typical fatal cases of staggers were produced in four out of seven cases.

This theory that toxic fungi cause forage-poisoning is not antagonistic to the facts in many of the more carefully observed outbreaks. The great variation in fungous growth

under different moisture conditions may explain the irregularity of the symptoms, as well as the occurrence of the disease under what may appear to be identical conditions. Many horses died as a result of eating mouldy baled hay, and as soon as this hay was eliminated the deaths ceased. Forage-poisoning, therefore, seems to be an auto-intoxication, due to certain chemical poisons or toxins formed by organismal activity.

Ruhl ('14), in a summary of the new theories concerning the etiology of pellagra, pointed out that investigators explain the casual relation of a corn diet to pellagra by four different physiological processes. However, these theories are inadequate, and serious objections are given to each. In this résumé we find that pellagra epidemics occur among people who have eaten corn that has been previously steamed, whereas persons in the same vicinity who have not eaten corn so treated were apparently not affected. It may be pointed out here that the steaming of corn before grinding will introduce conditions of moisture that favor the development of fungi, particularly *Rhizopus nigricans* and certain species of *Aspergillus*. In a moist condition the corn will become infected with fungi in a few days time; and after the corn has been dried, this fungous growth would no doubt pass unnoticed by the Italian peasants.

In the consideration of cryptococci, we do not find indications of toxic substances produced by these organisms. Galeotti and Pentimalli ('10) investigated the action of yeast toxin on the tissues of higher animals with three cultures of cryptococci obtained from tumors. The injection of the filtrate from liquid cultures or the injection of dead cultures gave negative results. However, the injection of living cells of certain cryptococci have proved fatal to rabbits, guinea-pigs, and dogs; and the organism seemed to show a selective action for the kidneys, the spleen, and the lungs. Loeb, Moore, and Fleisher ('13) were unable to find an extracellular toxin in cultures of the yeast-like fungus obtained from a carcinoma tissue. Death of the animals in this latter case was due to the rapid multiplication of yeast

cells in certain organs, more particularly the kidneys, which resulted in the blocking of the glomeruli and mechanical injury of the tissues. Cases of cryptomycoses in man have been reported in large numbers and appear to be less rare than they were formerly supposed.

Diseases of the skin are observed chiefly among persons living under conditions of uncleanness, or among those who combine such conditions with a tendency to profuse perspiration. The disease does not penetrate into the skin itself, but consists, as Plaut has pointed out, of a simple saprophytism of the inciting agent upon the skin.

Certain species of *Oospora* and *Sporotrichum* which occur as skin parasites on man are non-pathogenic for other animals. Quincke obtained negative results with the spores of *Oospora* (*Achorion*) by subcutaneous, intraperitoneal, or intravenous injections into mice, rabbits, and dogs. Citron ('05) made intraperitoneal injections of 14-day growths of *Oospora* in beer-wort suspended in salt solution. Pseudo-tuberculosis of the peritoneum resulted from injections of either the living or the heat-sterilized fungus.

The question whether or not there are any species among the many known yeasts which are pathogenic for man and animals has been the subject of observation for some time and has been answered mostly in the negative. All the animal experiments made with true yeasts found in nature have given negative results. Raum ('91) and Neumayer ('91), working on the pathogenicity of yeasts up to the year 1891, came to the conclusion that cultivated yeasts are non-injurious to animals. Raum used ten fungi, including *Saccharomyces cerevisiae*, *S. Pastorianus*, *S. ellipsoideus*, and *S. turbidans*. In only one case of Neumayer's was an injurious effect on animals manifested. Fischer and Brebeck ('94) obtained negative results with ten species of *Mycoderma*, *Monilia candida* Hansen, and *Torula salmonicolor*. Rabinowitsch ('95), with fifty cultures of non-spore-forming yeasts, obtained evidence of an injurious action with seven of these fungi, but only in white rats, and in these cases it was necessary to use large quantities. San Felice has been able to

find but one species of *Torula* that is pathogenic for animals. Cao ('00) obtained positive results with nine of forty-one cultures of what he called *Oidium*. These results of Cao appear doubtful, for in many instances where the organisms were regained from the inoculated animals they were present in the brain. It is possible for fungi to live saprophytically in the intestines and after death spread to all parts of the body by a rapid growth in the blood vessels. These results of Cao, therefore, must be questioned as to their validity.

Cultures of the twelve yeast-like fungi considered in this paper did not produce death in animals or the formation of lesions. Only three of the fungi were isolated from inoculated animals; and in these cases the organism was found in the intestines, and in only one animal was the inoculated organism found in the lungs. The organism found in the lungs of a guinea-pig may have developed in the blood vessels after death. A repetition with inoculations of these same organisms gave negative results.

CONCLUSIONS

From a review of the literature on animal pathology we find that fungi—not including bacteria—are of secondary importance in the formation of lesions in animal bodies and usually appear secondarily in infected tissues.

On the other hand, certain fungi are to be considered of primary importance in those instances in which they prove toxic to animals if consumed in large quantities on infected foods.

One hundred and eighty cultures of yeast-like fungi were obtained from 850 different sources, but only twelve of these grow on a blood-serum medium at 37°C.

These twelve fungi include six cultures of *Torula*, five of *Alternaria*, and one of *Oospora*.

One culture of *Torula* and four of *Alternaria* produced small quantities of alcohol in sugar nutrient solutions.

In acid yeast-water solutions all twelve of these organisms bring about a change in the acidity reaction, there being a decrease in the acidity of all the acid nutrient solutions

except in the presence of acetic acid and in culture No. 4.

The results of thirty-four animal experiments were negative, in that the death of certain animals was not caused by the formation of lesions or abnormalities due to the organisms injected.

No extracellular toxins were obtained from the cultures of these twelve yeast-like organisms.

The results of these experiments and a review of literature on animal pathology indicate either that pathogenic yeast-like fungi do not occur in nature, or that if they are present, they are so few as to be met with only under exceptional conditions.

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